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|---|--------------------------|--|--------------------------|----|---------|--------------------------|----|---------|------------------------|----|--|
| (51) International Patent Classification ⁵ : A61K 39/12, C07H 21/04 C07K 7/06, 7/08, 7/10 C07K 13/00, 15/28 | A1 | (11) International Publication Number: WO 90/11089 (43) International Publication Date: 4 October 1990 (04.10.90) | | | | | | | | | |
| (21) International Application Number: PCT/US90/01348 (22) International Filing Date: 15 March 1990 (15.03.90) (30) Priority data: <table border="0" style="width: 100%;"><tr><td style="width: 30%;">325,338</td><td style="width: 40%;">17 March 1989 (17.03.89)</td><td style="width: 30%;">US</td></tr><tr><td>341,334</td><td>20 April 1989 (20.04.89)</td><td>US</td></tr><tr><td>355,002</td><td>18 May 1989 (18.05.89)</td><td>US</td></tr></table> (71) Applicant: CHIRON CORPORATION [US/US]; 4560 Horton Street, Emeryville, CA 94608 (US). (72) Inventors: HOUGHTON, Michael ; 53 Rosemead Court, Danville, CA 94526 (US). CHOO, Qui-Lim ; 5700 Fern Street, El Cerrito, CA 94530 (US). KUO, George ; 1370 Sixth Avenue, San Francisco, CA 94122 (US). | | 325,338 | 17 March 1989 (17.03.89) | US | 341,334 | 20 April 1989 (20.04.89) | US | 355,002 | 18 May 1989 (18.05.89) | US | (74) Agents: MONROY, Gladys, H. et al. ; Irell & Manella, 545 Middlefield Road, Suite 200, Menlo Park, CA 94025 (US). (81) Designated States: AU, FI, HU, JP, KR, NO, SU. Published <i>With international search report.</i> |
| 325,338 | 17 March 1989 (17.03.89) | US | | | | | | | | | |
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| 355,002 | 18 May 1989 (18.05.89) | US | | | | | | | | | |
| (54) Title: NANBV DIAGNOSTICS AND VACCINES (57) Abstract <p>A new virus, Hepatitis C virus (HCV), which has proven to be the major etiologic agent of blood-borne NANBH, was discovered by Applicant. The initial work on this virus, which includes a partial genomic sequence of the prototype HCV isolate, is described in EPO Pub. No. 318,216, and PCT Pub. No. WO 89/04669. The present invention, which in part is based on new HCV sequences and polypeptides which are not disclosed in the above-cited publications, includes the application of these new sequences and polypeptides in immunoassays, probe diagnostics, anti-HCV antibody production, PCR technology, and recombinant DNA technology. Included within the invention also are novel, immunogenic polypeptides encoded within clones containing HCV cDNA, novel methods for purifying an immunogenic HCV polypeptide, and antisense polynucleotides derived from HCV cDNA.</p> | | | | | | | | | | | |

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NANBV DIAGNOSTICS AND VACCINES

10

Technical Field

The invention relates to materials and methodologies for managing the spread of non-A, non-B hepatitis virus (NANBV) infection. More specifically, it relates to polynucleotides derived from the genome of an etiologic agent of NANBH, hepatitis C virus (HCV), to polypeptides encoded therein, and to antibodies directed to the polypeptides. These reagents are useful as screening agents for HCV and its infection, and as protective agents against the disease.

20

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Cited Patents

5 EPO Pub. No. 318,216
PCT Pub. No. WO 89/04669
U.S. Patent No. 4,341,761
U.S. Patent No. 4,399,121
U.S. Patent No. 4,427,783
U.S. Patent No. 4,444,887
10 U.S. Patent No. 4,466,917
U.S. Patent No. 4,472,500
U.S. Patent No. 4,491,632
U.S. Patent No. 4,493,890

Background Art

15 Non-A, Non-B hepatitis (NANBH) is a transmissible disease or family of diseases that are believed to be viral-induced, and that are distinguishable from other forms of viral-associated liver diseases, including that caused by the known hepatitis viruses,
20 i.e., hepatitis A virus (HAV), hepatitis B virus (HBV), and delta hepatitis virus (HDV), as well as the hepatitis induced by cytomegalovirus (CMV) or Epstein-Barr virus (EBV). NANBH was first identified in transfused individuals. Transmission from man to chimpanzee and se-
25 rial passage in chimpanzees provided evidence that NANBH is due to a transmissible infectious agent or agents.

Epidemiologic evidence is suggestive that there may be three types of NANBH: the water-borne epidemic type; the blood or needle associated type; and the
30 sporadically occurring (community acquired) type. However, the number of agents which may be the causative of NANBH are unknown.

Clinical diagnosis and identification of NANBH has been accomplished primarily by exclusion of other
35 viral markers. Among the methods used to detect putative NANBV antigens and antibodies are agar-gel diffusion,

counterimmunoelectrophoresis, immunofluorescence
microscopy, immune electron microscopy, radioimmunoassay,
and enzyme-linked immunosorbent assay. However, none of
5 these assays has proved to be sufficiently sensitive,
specific, and reproducible to be used as a diagnostic test
for NANBH.

Previously there was neither clarity nor agree-
ment as to the identity or specificity of the antigen
10 antibody systems associated with agents of NANBH. This
was due, at least in part, to the prior or co-infection of
HBV with NANBV in individuals, and to the known complexity
of the soluble and particulate antigens associated with
HBV, as well as to the integration of HBV DNA into the
15 genome of liver cells. In addition, there is the pos-
sibility that NANBH is caused by more than one infectious
agent, as well as the possibility that NANBH has been mis-
diagnosed. Moreover, it is unclear what the serological
assays detect in the serum of patients with NANBH. It has
20 been postulated that the agar-gel diffusion and counter-
immunoelectrophoresis assays detect autoimmune responses
or nonspecific protein interactions that sometimes occur
between serum specimens, and that they do not represent
specific NANBV antigen-antibody reactions. The
25 immunofluorescence, and enzyme-linked immunosorbent, and
radioimmunoassays appear to detect low levels of a
rheumatoid-factor-like material that is frequently present
in the serum of patients with NANBH as well as in patients
with other hepatic and nonhepatic diseases. Some of the
30 reactivity detected may represent antibody to host-
determined cytoplasmic antigens.

There have been a number of candidate NANBV.
See, for example the reviews by Prince (1983), Feinstone
and Hoofnagle (1984), and Overby (1985, 1986, 1987) and
35 the article by Iwarson (1987). However, there is no proof

that any of these candidates represent the etiological agent of NANBH.

5 The demand for sensitive, specific methods for screening and identifying carriers of NANBV and NANBV contaminated blood or blood products is significant. Post-transfusion hepatitis (PTH) occurs in approximately 10% of transfused patients, and NANBH accounts for up to 90% of these cases. The major problem in this disease is
10 the frequent progression to chronic liver damage (25-55%).

 Patient care as well as the prevention of transmission of NANBH by blood and blood products or by close personal contact require reliable screening,
15 diagnostic and prognostic tools to detect nucleic acids, antigens and antibodies related to NANBV. In addition, there is also a need for effective vaccines and
 immunotherapeutic therapeutic agents for the prevention and/or treatment of the disease.

20 Applicant discovered a new virus, the Hepatitis C virus (HCV), which has proven to be the major etiologic agent of blood-borne NANBH (BB-NANBH). Applicant's initial work, including a partial genomic sequence of the prototype HCV isolate, CDC/HCV1 (also called HCV1), is described in EPO Pub. No. 318,216 (published 31 May 1989)
25 and PCT Pub. No. WO 89/04669 (published 1 June 1989). The disclosures of these patent applications, as well as any corresponding national patent applications, are incorporated herein by reference. These applications teach, inter alia, recombinant DNA methods of cloning and
30 expressing HCV sequences, HCV polypeptides, HCV immunodiagnostic techniques, HCV probe diagnostic techniques, anti-HCV antibodies, and methods of isolating new hCV sequences, including sequences of new HCV
 isolates.

35

Disclosure of the Invention

5 The present invention is based, in part, on new HCV sequences and polypeptides that are not disclosed in EPO Pub. No. 318,216, or in PCT Pub. No. WO 89/04669. Included within the invention is the application of these new sequences and polypeptides in, inter alia, immunodiagnostics, probe diagnostics, anti-HCV antibody production, PCR technology and recombinant DNA technology. 10 Included within the invention, also, are new immunoassays based upon the immunogenicity of HCV polypeptides disclosed herein. The new subject matter claimed herein, while developed using techniques described in, for example, EPO Pub. No. 318,216, has a priority date which 15 antecedes that publication, or any counterpart thereof. Thus, the invention provides novel compositions and methods useful for screening samples for HCV antigens and antibodies, and useful for treatment of HCV infections.

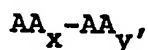
20 Accordingly, one aspect of the invention is a recombinant polynucleotide comprising a sequence derived from HCV cDNA, wherein the HCV cDNA is in clone 13i, or clone 26j, or clone 59a, or clone 84a, or clone CA156e, or clone 167b, or clone pil4a, or clone CA216a, or clone CA290a, or clone ag30a, or clone 205a, or clone 18g, or 25 clone 16jh, or wherein the HCV cDNA is of a sequence indicated by nucleotide numbers -319 to 1348 or 8659 to 8866 in Fig. 17.

30 Another aspect of the invention is a purified polypeptide comprising an epitope encoded within HCV cDNA wherein the HCV cDNA is of a sequence indicated by nucleotide numbers -319 to 1348 or 8659 to 8866 in Fig. 17.

35 Yet another aspect of the invention is an immunogenic polypeptide produced by a cell transformed with a recombinant expression vector comprising an ORF of DNA derived from HCV cDNA, wherein the HCV cDNA is comprised

of a sequence derived from the HCV cDNA sequence in clone
 CA279a, or clone CA74a, or clone 13i, or clone CA290a, or
 clone 33C or clone 40b, or clone 33b, or clone 25c, or
 5 clone 14c, or clone 8f, or clone 33f, or clone 33g, or
 clone 39c, or clone 15e, and wherein the ORF is operably
 linked to a control sequence compatible with a desired
 host.

Another aspect of the invention is a peptide
 10 comprising an HCV epitope, wherein the peptide is of the
 formula



15 wherein x and y designate amino acid numbers shown in Fig.
 17, and wherein the peptide is selected from the group
 consisting of AA1-AA25, AA1-AA50, AA1-AA84, AA9-AA177,
 AA1-AA10, AA5-AA20, AA20-AA25, AA35-AA45, AA50-AA100,
 AA40-AA90, AA45-AA65, AA65-AA75, AA80-90, AA99-AA120,
 20 AA95-AA110, AA105-AA120, AA100-AA150, AA150-AA200,
 AA155-AA170, AA190-AA210, AA200-AA250, AA220-AA240,
 AA245-AA265, AA250-AA300, AA290-AA330, AA290-305,
 AA300-AA350, AA310-AA330, AA350-AA400, AA380-AA395,
 AA405-AA495, AA400-AA450, AA405-AA415, AA415-AA425,
 25 AA425-AA435, AA437-AA582, AA450-AA500, AA440-AA460,
 AA460-AA470, AA475-AA495, AA500-AA550, AA511-AA690, AA515-
 AA550, AA550-AA600, AA550-AA625, AA575-AA605, AA585-AA600,
 AA600-AA650, AA600-AA625, AA635-AA665, AA650-AA700,
 AA645-AA680, AA700-AA750, AA700-AA725, AA700-AA750,
 30 AA725-AA775, AA770-AA790, AA750-AA800, AA800-AA815,
 AA825-AA850, AA850-AA875, AA800-AA850, AA920-AA990,
 AA850-AA900, AA920-AA945, AA940-AA965, AA970-AA990,
 AA950-AA1000, AA1000-AA1060, AA1000-AA1025, AA1000-AA1050,
 AA1025-AA1040, AA1040-AA1055, AA1075-AA1175,
 35 AA1050-AA1200, AA1070-AA1100, AA1100-AA1130,
 AA1140-AA1165, AA1192-AA1457, AA1195-AA1250,

AA1200-AA1225, AA1225-AA1250, AA1250-AA1300,
AA1260-AA1310, AA1260-AA1280, AA1266-AA1428,
5 AA1300-AA1350, AA1290-AA1310, AA1310-AA1340, AA1345-
AA1405, AA1345-AA1365, AA1350-AA1400, AA1365-AA1380,
AA1380-AA1405, AA1400-AA1450, AA1450-AA1500,
AA1460-AA1475, AA1475-AA1515, AA1475-AA1500,
AA1500-AA1550, AA1500-AA1515, AA1515-AA1550,
10 AA1550-AA1600, AA1545-AA1560, AA1569-AA1931,
AA1570-AA1590, AA1595-AA1610, AA1590-AA1650,
AA1610-AA1645, AA1650-AA1690, AA1685-AA1770,
AA1689-AA1805, AA1690-AA1720, AA1694-AA1735,
AA1720-AA1745, AA1745-AA1770, AA1750-AA1800,
15 AA1775-AA1810, AA1795-AA1850, AA1850-AA1900,
AA1900-AA1950, AA1900-AA1920, AA1916-AA2021,
AA1920-AA1940, AA1949-AA2124, AA1950-AA2000,
AA1950-AA1985, AA1980-AA2000, AA2000-AA2050,
AA2005-AA2025, AA2020-AA2045, AA2045-AA2100,
20 AA2045-AA2070, AA2054-AA2223, AA2070-AA2100,
AA2100-AA2150, AA2150-AA2200, AA2200-AA2250,
AA2200-AA2325, AA2250-AA2330, AA2255-AA2270,
AA2265-AA2280, AA2280-AA2290, AA2287-AA2385,
AA2300-AA2350, AA2290-AA2310, AA2310-AA2330,
AA2330-AA2350, AA2350-AA2400, AA2348-AA2464,
25 AA2345-AA2415, AA2345-AA2375, AA2370-AA2410,
AA2371-AA2502, AA2400-AA2450, AA2400-AA2425,
AA2415-AA2450, AA2445-AA2500, AA2445-AA2475,
AA2470-AA2490, AA2500-AA2550, AA2505-AA2540,
AA2535-AA2560, AA2550-AA2600, AA2560-AA2580,
30 AA2600-AA2650, AA2605-AA2620, AA2620-AA2650,
AA2640-AA2660, AA2650-AA2700, AA2655-AA2670,
AA2670-AA2700, AA2700-AA2750, AA2740-AA2760,
AA2750-AA2800, AA2755-AA2780,
AA2780-AA2830, AA2785-AA2810, AA2796-AA2886,
35 AA2810-AA2825, AA2800-AA2850, AA2850-AA2900,

AA2850-AA2865, AA2885-AA2905, AA2900-AA2950,
AA2910-AA2930, AA2925-AA2950, AA2945-end(C' terminal).

5 Still another aspect of the invention is a
monoclonal antibody directed against an epitope encoded in
HCV cDNA, wherein the HCV cDNA is of a sequence indicated
by nucleotide numbers -319 to 1348 or 8659 to 8866 in Fig.
17, or is the sequence present in clone 13i, or clone 26j,
or clone 59a, or clone 84a, or clone CA156e, or clone
10 167b, or clone pil4a, or clone CA216a, or clone CA290a, or
clone ag30a, or clone 205a, or clone 18g, or clone 16jh.

Yet another aspect of the invention is a
preparation of purified polyclonal antibodies directed
against a polypeptide comprised of an epitope encoded
15 within HCV cDNA, wherein the HCV cDNA is of a sequence
indicated by nucleotide numbers -319 to 1348 or 8659 to
8866 in Fig. 17, or is the sequence present in in clone
13i, or clone 26j, or clone 59a, or clone 84a, or clone
CA156e, or clone 167b, or clone pil4a, or clone CA216a, or
20 clone CA290a, or clone ag30a, or clone 205a, or clone 18g,
or clone 16jh.

Still another aspect of the invention is a
polynucleotide probe for HCV, wherein the probe is
comprised of an HCV sequence derived from an HCV cDNA
25 sequence indicated by nucleotide numbers -319 to 1348 or
8659 to 8866 in Fig. 17, or from the complement of the HCV
cDNA sequence.

Yet another aspect of the invention is a kit for
analyzing samples for the presence of polynucleotides from
30 HCV comprising a polynucleotide probe containing a
nucleotide sequence of about 8 or more nucleotides,
wherein the nucleotide sequence is derived from HCV cDNA
which is of a sequence indicated by nucleotide numbers -
319 to 1348 or 8659 to 8866 in Fig. 17, wherein the
35 polynucleotide probe is in a suitable container.

Another aspect of the invention is a kit for analyzing samples for the presence of an HCV antigen comprising an antibody which reacts immunologically with
5 an HCV antigen, wherein the antigen contains an epitope encoded within HCV cDNA which is of a sequence indicated by nucleotide numbers -319 to 1348 or 8659 to 8866 in Fig. 17, or wherein the HCV cDNA is in clone 13i, or clone 26j, or clone 59a, or clone 84a, or clone CA156e, or clone
10 167b, or clone pil4a, or clone CA216a, or clone CA290a, or clone ag30a, or clone 205a, or clone 18g, or clone 16jh.

Yet another aspect of the invention is a kit for analyzing samples for the presence of an HCV antibody comprising an antigenic polypeptide containing an HCV
15 epitope encoded within HCV cDNA which is of a sequence indicated by nucleotide numbers -319 to 1348 or 8659 to 8866 in Fig. 17, or is in clone 13i, or clone 26j, or clone 59a, or clone 84a, or clone CA156e, or clone 167b, or clone pil4a, or clone CA216a, or clone CA290a, or clone
20 ag30a, or clone 205a, or clone 18g, or clone 16jh.

Another aspect of the invention is a kit for analyzing samples for the presence of an HCV antibody comprising an antigenic polypeptide expressed from HCV
25 cDNA in clone CA279a, or clone CA74a, or clone 13i, or clone CA290a, or clone 33C or clone 40b, or clone 33b, or clone 25c, or clone 14c, or clone 8f, or clone 33f, or clone 33g, or clone 39c, or clone 15e, wherein the antigenic polypeptide is present in a suitable container.

Still another aspect of the invention is a
30 method for detecting HCV nucleic acids in a sample comprising:

(a) reacting nucleic acids of the sample with a polynucleotide probe for HCV, wherein the probe is
35 comprised of an HCV sequence derived from an HCV cDNA sequence is of a sequence indicated by nucleotide numbers -319 to 1348 or 8659 to 8866 in Fig. 17, and wherein the

reacting is under conditions which allow the formation of a polynucleotide duplex between the probe and the HCV nucleic acid from the sample; and (b) detecting a
5 polynucleotide duplex which contains the probe, formed in step (a).

Yet another aspect of the invention is an immunoassay for detecting an HCV antigen comprising:

10 (a) incubating a sample suspected of containing an HCV antigen with an antibody directed against an HCV epitope encoded in HCV cDNA, wherein the HCV cDNA is of a sequence indicated by nucleotide numbers -319 to 1348 or 8659 to 8866 in Fig. 17, or is the sequence present in
15 clone 13i, or clone 26j, or clone 59a, or clone 84a, or clone CA156e, or clone 167b, or clone pil4a, or clone CA216a, or clone CA290a, or clone ag30a, or clone 205a, or clone 18g, or clone 16jh, and wherein the incubating is under conditions which allow formation of an antigen-
20 antibody complex; and (b) detecting an antibody-antigen complex formed in step (a) which contains the antibody.

Still another aspect of the invention is an immunoassay for detecting antibodies directed against an HCV antigen comprising:

25 (a) incubating a sample suspected of containing anti-HCV antibodies with an antigen polypeptide containing an epitope encoded in HCV cDNA, wherein the HCV cDNA is of a sequence indicated by nucleotide numbers -319 to 1348 or 8659 to 8866 in Fig. 17, or is the sequence present in
30 clone 13i, or clone 26j, or clone 59a, or clone 84a, or clone CA156e, or clone 167b, or clone pil4a, or clone CA216a, or clone CA290a, or clone ag30a, or clone 205a, or clone 18g, or clone 16jh, and wherein the incubating is under conditions which allow formation of an antigen-
35 antibody complex; and detecting an antibody-antigen complex formed in step (a) which contains the antigen polypeptide.

Another aspect of the invention is a vaccine for treatment of HCV infection comprising an immunogenic polypeptide containing an HCV epitope encoded in HCV cDNA, wherein the HCV cDNA is of a sequence indicated by nucleotide numbers -319 to 1348 or 8659 to 8866 in Fig. 17 or is the sequence present in clone 13i, or clone 26j, or clone 59a, or clone 84a, or clone CA156e, or clone 167b, or clone pi14a, or clone CA216a, or clone CA290a, or clone ag30a, or clone 205a, or clone 18g, or clone 16jh, and wherein the immunogenic polypeptide is present in a pharmacologically effective dose in a pharmaceutically acceptable excipient.

Yet another aspect of the invention is a method for producing antibodies to HCV comprising administering to an individual an isolated immunogenic polypeptide containing an HCV epitope encoded in HCV cDNA, wherein the HCV cDNA is of a sequence indicated by nucleotide numbers -319 to 1348 or 8659 to 8866 in Fig. 17, or is of the sequence present in clone CA279a, or clone CA74a, or clone 13i, or clone CA290a, or clone 33C or clone 40b, or clone 33b, or clone 25c, or clone 14c, or clone 8f, or clone 33f, or clone 33g, or clone 39c, or clone 15e, and wherein the immunogenic polypeptide is present in a pharmacologically effective dose in a pharmaceutically acceptable excipient.

Still another aspect of the invention is an antisense polynucleotide derived from HCV cDNA, wherein the HCV cDNA is that shown in Fig. 17.

Yet another aspect of the invention is a method for preparing purified fusion polypeptide C100-3 comprising:

(a) providing a crude cell lysate containing polypeptide C100-3,

- (b) treating the crude cell lysate with an amount of acetone which causes the polypeptide to precipitate,
- 5 (c) isolating and solubilizing the precipitated material,
- (d) isolating the C100-3 polypeptide by anion exchange chromatography, and
- 10 (e) further isolating the C100-3 polypeptide of step (d) by gel filtration.

Brief Description of the Drawings

15 Fig. 1 shows the sequence of the HCV cDNA in clone 12f, and the amino acids encoded therein.

Fig. 2 shows the HCV cDNA sequence in clone k9-1, and the amino acids encoded therein.

20 Fig. 3 shows the sequence of clone 15e, and the amino acids encoded therein.

Fig. 4 shows the nucleotide sequence of HCV cDNA in clone 13i, the amino acids encoded therein, and the sequences which overlap with clone 12f.

25 Fig. 5 shows the nucleotide sequence of HCV cDNA in clone 26j, the amino acids encoded therein, and the sequences which overlap clone 13i.

Fig. 6 shows the nucleotide sequence of HCV cDNA in clone CA59a, the amino acids encoded therein, and the sequences which overlap with clones 26j and K9-1.

30 Fig. 7 shows the nucleotide sequence of HCV cDNA in clone CA84a, the amino acids encoded therein, and the sequences which overlap with clone CA59a.

35 Fig. 8 shows the nucleotide sequence of HCV cDNA in clone CA156e, the amino acids encoded therein, and the sequences which overlap with CA84a.

Fig. 9 shows the nucleotide sequence of HCV cDNA in clone CA167b, the amino acids encoded therein, and the sequences which overlap CA156e.

Fig. 10 shows the nucleotide sequence of HCV cDNA in clone CA216a, the amino acids encoded therein, and the overlap with clone CA167b.

Fig. 11 shows the nucleotide sequence of HCV cDNA in clone CA290a, the amino acids encoded therein, and the overlap with clone CA216a.

Fig. 12 shows the nucleotide sequence of HCV cDNA in clone ag30a and the overlap with clone CA290a.

Fig. 13 shows the nucleotide sequence of HCV cDNA in clone CA205a, and the overlap with the HCV cDNA sequence in clone CA290a.

Fig. 14 shows the nucleotide sequence of HCV cDNA in clone 18g, and the overlap with the HCV cDNA sequence in clone ag30a.

Fig. 15 shows the nucleotide sequence of HCV cDNA in clone 16jh, the amino acids encoded therein, and the overlap of nucleotides with the HCV cDNA sequence in clone 15e.

Fig. 16 shows the ORF of HCV cDNA derived from clones pil4a, CA167b, CA156e, CA84a, CA59a, K9-1, 12f, 14i, 11b, 7f, 7e, 8h, 33c, 40b, 37b, 35, 36, 81, 32, 33b, 25c, 14c, 8f, 33f, 33g, 39c, 35f, 19g, 26g, and 15e.

Fig. 17 shows the sense strand of the compiled HCV cDNA sequence derived from the above-described clones and the compiled HCV cDNA sequence published in EPO Pub. No. 318,216. The clones from which the sequence was derived are b114a, 18g, ag30a, CA205a, CA290a, CA216a, pil4a, CA167b, CA156e, CA84a, CA59a, K9-1 (also called k9-1), 26j, 13i, 12f, 14i, 11b, 7f, 7e, 8h, 33c, 40b, 37b, 35, 36, 81, 32, 33b, 25c, 14c, 8f, 33f, 33g, 39c, 35f, 19g, 26g, 15e, b5a, and 16jh. In the figure the three horizontal dashes above the sequence indicate the position

of the putative initiator methionine codon; the two vertical dashes indicate the first and last nucleotides of the published sequence. Also shown in the figure is the amino acid sequence of the putative polyprotein encoded in the HCV cDNA.

Fig. 18 is a diagram of the immunological colony screening method used in antigenic mapping studies.

Fig. 19 shows the hydrophobicity profiles of polyproteins encoded in HCV and in West Nile virus.

Fig. 20 is a tracing of the hydrophilicity/hydrophobicity profile and of the antigenic index of the putative HCV polyprotein.

Fig. 21 shows the conserved co-linear peptides in HCV and Flaviviruses.

Modes for Carrying Out the Invention

20 I. Definitions

The term "hepatitis C virus" has been reserved by workers in the field for an heretofore unknown etiologic agent of NANBH. Accordingly, as used herein, "hepatitis C virus" (HCV) refers to an agent causative of NANBH, which was formerly referred to as NANBV and/or BB-NANBV. The terms HCV, NANBV, and BB-NANBV are used interchangeably herein. As an extension of this terminology, the disease caused by HCV, formerly called NANB hepatitis (NANBH), is called hepatitis C. The terms NANBH and hepatitis C may be used interchangeably herein.

The term "HCV", as used herein, denotes a viral species of which pathogenic strains cause NANBH, and attenuated strains or defective interfering particles derived therefrom. As shown infra., the HCV genome is comprised of RNA. It is known that RNA containing viruses have relatively high rates of spontaneous mutation, i.e., reportedly on the order of 10^{-3} to 10^{-4} per incorporated

nucleotide (Fields & Knipe (1986)). Therefore, there are multiple strains, which may be virulent or avirulent, within the HCV species described infra. The compositions and methods described herein, enable the propagation, identification, detection, and isolation of the various HCV strains or isolates. Moreover, the disclosure herein allows the preparation of diagnostics and vaccines for the various strains, as well as compositions and methods that have utility in screening procedures for anti-viral agents for pharmacologic use, such as agents that inhibit replication of HCV.

The information provided herein, although derived from the prototype strain or isolate of HCV, hereinafter referred to as CDC/HCV1 (also called HCV1), is sufficient to allow a viral taxonomist to identify other strains which fall within the species. The information provided herein allows the belief that HCV is a Flavi-like virus. The morphology and composition of Flavivirus particles are known, and are discussed in Brinton (1986). Generally, with respect to morphology, Flaviviruses contain a central nucleocapsid surrounded by a lipid bilayer. Virions are spherical and have a diameter of about 40-50 nm. Their cores are about 25-30 nm in diameter. Along the outer surface of the virion envelope are projections that are about 5-10 nm long with terminal knobs about 2 nm in diameter.

Different strains or isolates of HCV are expected to contain variations at the amino acid and nucleic acids compared with the prototype isolate, HCV1. Many isolates are expected to show much (i.e. more than about 40%) homology in the total amino acid sequence compared with HCV1. However, it may also be found that other less homologous HCV isolates. These would be defined as HCV strains according to various criteria such as an ORF of approximately 9,000 nucleotides to ap-

proximately 12,000 nucleotides, encoding a polyprotein similar in size to that of HCV1, an encoded polyprotein of similar hydrophobic and antigenic character to that of HCV1, and the presence of co-linear peptide sequences that are conserved with HCV1. In addition, the genome would be a positive-stranded RNA.

HCV encodes at least one epitope which is immunologically identifiable with an epitope in the HCV genome from which the cDNAs described herein are derived; preferably the epitope is contained an amino acid sequence described herein. The epitope is unique to HCV when compared to other known Flaviviruses. The uniqueness of the epitope may be determined by its immunological reactivity with anti-HCV antibodies and lack of immunological reactivity with antibodies to other Flavivirus species. Methods for determining immunological reactivity are known in the art, for example, by radioimmunoassay, by Elisa assay, by hemagglutination, and several examples of suitable techniques for assays are provided herein.

In addition to the above, the following parameters of nucleic acid homology and amino acid homology are applicable, either alone or in combination, in identifying a strain or isolate as HCV. Since HCV strains and isolates are evolutionarily related, it is expected that the overall homology of the genomes at the nucleotide level probably will be about 40% or greater, probably about 60% or greater, and even more probably about 80% or greater; and in addition that there will be corresponding contiguous sequences of at least about 13 nucleotides. The correspondence between the putative HCV strain genomic sequence and the CDC/HCV1 cDNA sequence can be determined by techniques known in the art. For example, they can be determined by a direct comparison of the sequence information of the polynucleotide from the putative HCV, and the HCV cDNA sequence(s) described herein. For example, also,

they can be determined by hybridization of the polynucleotides under conditions which form stable duplexes between homologous regions (for example, those
5 which would be used prior to S_1 digestion), followed by digestion with single stranded specific nuclease(s), followed by size determination of the digested fragments.

Because of the evolutionary relationship of the strains or isolates of HCV, putative HCV strains or
10 isolates are identifiable by their homology at the polypeptide level. Generally, HCV strains or isolates are expected to be more than about 40% homologous, probably more than about 70% homologous, and even more probably
15 more than about 80% homologous, and some may even be more than about 90% homologous at the polypeptide level. The techniques for determining amino acid sequence homology are known in the art. For example, the amino acid
sequence may be determined directly and compared to the sequences provided herein. Alternatively the nucleotide
20 sequence of the genomic material of the putative HCV may be determined (usually via a cDNA intermediate), the amino acid sequence encoded therein can be determined, and the corresponding regions compared.

As used herein, a polynucleotide "derived from"
25 a designated sequence refers to a polynucleotide sequence which is comprised of a sequence of approximately at least about 6 nucleotides, preferably at least about 8 nucleotides, more preferably at least about 10-12
nucleotides, and even more preferably at least about 15-20
30 nucleotides corresponding to a region of the designated nucleotide sequence. "Corresponding" means homologous to or complementary to the designated sequence. Preferably, the sequence of the region from which the polynucleotide is derived is homologous to or complementary to a sequence
35 which is unique to an HCV genome. Whether or not a sequence is unique to the HCV genome can be determined by

techniques known to those of skill in the art. For example, the sequence can be compared to sequences in databanks, e.g., Genbank, to determine whether it is present in the uninfected host or other organisms. The sequence can also be compared to the known sequences of other viral agents, including those which are known to induce hepatitis, e.g., HAV, HBV, and HDV, and to other members of the Flaviviridae. The correspondence or non-correspondence of the derived sequence to other sequences can also be determined by hybridization under the appropriate stringency conditions. Hybridization techniques for determining the complementarity of nucleic acid sequences are known in the art, and are discussed infra. See also, for example, Maniatis et al. (1982). In addition, mismatches of duplex polynucleotides formed by hybridization can be determined by known techniques, including for example, digestion with a nuclease such as S1 that specifically digests single-stranded areas in duplex polynucleotides. Regions from which typical DNA sequences may be "derived" include but are not limited to, for example, regions encoding specific epitopes, as well as non-transcribed and/or non-translated regions.

The derived polynucleotide is not necessarily physically derived from the nucleotide sequence shown, but may be generated in any manner, including for example, chemical synthesis or DNA replication or reverse transcription or transcription. In addition, combinations of regions corresponding to that of the designated sequence may be modified in ways known in the art to be consistent with an intended use.

Similarly, a polypeptide or amino acid sequence "derived from" a designated nucleic acid sequence refers to a polypeptide having an amino acid sequence identical to that of a polypeptide encoded in the sequence, or a portion thereof wherein the portion consists of at least

3-5 amino acids, and more preferably at least 8-10 amino acids, and even more preferably at least 11-15 amino acids, or which is immunologically identifiable with a polypeptide encoded in the sequence.

A recombinant or derived polypeptide is not necessarily translated from a designated nucleic acid sequence, for example, the HCV cDNA sequences described herein, or from an HCV genome; it may be generated in any manner, including for example, chemical synthesis, or expression of a recombinant expression system, or isolation from mutated HCV. A recombinant or derived polypeptide may include one or more analogs of amino acids or unnatural amino acids in its sequence. Methods of inserting analogs of amino acids into a sequence are known in the art. It also may include one or more labels, which are known to those of skill in the art.

The term "recombinant polynucleotide" as used herein intends a polynucleotide of genomic, cDNA, semisynthetic, or synthetic origin which, by virtue of its origin or manipulation which: (1) is not associated with all or a portion of a polynucleotide with which it is associated in nature, (2) is linked to a polynucleotide other than that to which it is linked in nature, or (3) does not occur in nature.

The term "polynucleotide" as used herein refers to a polymeric form of nucleotides of any length, either ribonucleotides or deoxyribonucleotides. This term refers only to the primary structure of the molecule. Thus, this term includes double- and single-stranded DNA, as well as double- and single stranded RNA. It also includes known types of modifications, for example, labels which are known in the art, methylation, "caps", substitution of one or more of the naturally occurring nucleotides with an analog, internucleotide modifications such as, for example, those with uncharged linkages (e.g., methyl

phosphonates, phosphotriesters, phosphoamidates, carbamates, etc.) and with charged linkages (e.g., phosphorothioates, phosphorodithioates, etc.), those
5 containing pendant moieties, such as, for example proteins (including for e.g., nucleases, toxins, antibodies, signal peptides, poly-L-lysine, etc.), those with intercalators (e.g., acridine, psoralen, etc.), those containing
10 chelators (e.g., metals, radioactive metals, boron, oxidative metals, etc.), those containing alkylators, those with modified linkages (e.g., alpha anomeric nucleic acids, etc.), as well as unmodified forms of the polynucleotide.

15 The term "purified viral polynucleotide" refers to an HCV genome or fragment thereof which is essentially free, i.e., contains less than about 50%, preferably less than about 70%, and even more preferably less than about 90% of polypeptides with which the viral polynucleotide is naturally associated. Techniques for purifying viral
20 polynucleotides from viral particles are known in the art, and include for example, disruption of the particle with a chaotropic agent, differential extraction and separation of the polynucleotide(s) and polypeptides by ion-exchange chromatography, affinity chromatography, and sedimentation
25 according to density.

The term "purified viral polypeptide" refers to an HCV polypeptide or fragment thereof which is essentially free, i.e., contains less than about 50%, preferably less than about 70%, and even more preferably
30 less than about 90%, of cellular components with which the viral polypeptide is naturally associated. Techniques for purifying viral polypeptides are known in the art, and examples of these techniques are discussed infra. The term
35 "purified viral polynucleotide" refers to an HCV genome or fragment thereof which is essentially free, i.e., contains less than about 20%, preferably less than about 50%, and

even more preferably less than about 70% of polypeptides with which the viral polynucleotide is naturally associated. Techniques for purifying viral polynucleotides from viral particles are known in the art, and include for example, disruption of the particle with a chaotropic agent, and separation of the polynucleotide(s) and polypeptides by ion-exchange chromatography, affinity chromatography, and sedimentation according to density.

"Recombinant host cells", "host cells", "cells", "cell lines", "cell cultures", and other such terms denoting microorganisms or higher eukaryotic cell lines cultured as unicellular entities refer to cells which can be, or have been, used as recipients for recombinant vector or other transfer DNA, and include the progeny of the original cell which has been transfected. It is understood that the progeny of a single parental cell may not necessarily be completely identical in morphology or in genomic or total DNA complement as the original parent, due to natural, accidental, or deliberate mutation.

A "replicon" is any genetic element, e.g., a plasmid, a chromosome, a virus, a cosmid, etc. that behaves as an autonomous unit of polynucleotide replication within a cell; i.e., capable of replication under its own control.

A "vector" is a replicon in which another polynucleotide segment is attached, so as to bring about the replication and/or expression of the attached segment.

"Control sequence" refers to polynucleotide sequences which are necessary to effect the expression of coding sequences to which they are ligated. The nature of such control sequences differs depending upon the host organism; in prokaryotes, such control sequences generally include promoter, ribosomal binding site, and terminators; in eukaryotes, generally, such control sequences include promoters, terminators and, in some instances, enhancers.

The term "control sequences" is intended to include, at a minimum, all components whose presence is necessary for expression, and may also include additional components whose presence is advantageous, for example, leader sequences.

"Operably linked" refers to a juxtaposition wherein the components so described are in a relationship permitting them to function in their intended manner. A control sequence "operably linked" to a coding sequence is ligated in such a way that expression of the coding sequence is achieved under conditions compatible with the control sequences.

An "open reading frame" (ORF) is a region of a polynucleotide sequence which encodes a polypeptide; this region may represent a portion of a coding sequence or a total coding sequence.

A "coding sequence" is a polynucleotide sequence which is transcribed into mRNA and/or translated into a polypeptide when placed under the control of appropriate regulatory sequences. The boundaries of the coding sequence are determined by a translation start codon at the 5'-terminus and a translation stop codon at the 3'-terminus. A coding sequence can include, but is not limited to mRNA, cDNA, and recombinant polynucleotide sequences.

"Immunologically identifiable with/as" refers to the presence of epitope(s) and polypeptides(s) which are also present in the designated polypeptide(s), usually HCV proteins. Immunological identity may be determined by antibody binding and/or competition in binding; these techniques are known to those of average skill in the art, and are also illustrated infra.

As used herein, "epitope" refers to an antigenic determinant of a polypeptide; an epitope could comprise 3 amino acids in a spatial conformation which is unique to

the epitope, generally an epitope consists of at least 5 such amino acids, and more usually, consists of at least 8-10 such amino acids. Methods of determining the spatial conformation of amino acids are known in the art, and include, for example, x-ray crystallography and 2-dimensional nuclear magnetic resonance.

A polypeptide is "immunologically reactive" with an antibody when it binds to an antibody due to antibody recognition of a specific epitope contained within the polypeptide. Immunological reactivity may be determined by antibody binding, more particularly by the kinetics of antibody binding, and/or by competition in binding using as competitor(s) a known polypeptide(s) containing an epitope against which the antibody is directed. The techniques for determining whether a polypeptide is immunologically reactive with an antibody are known in the art.

As used herein, the term "immunogenic polypeptide" is a polypeptide that elicits a cellular and/or humoral response, whether alone or linked to a carrier in the presence or absence of an adjuvant.

The term "polypeptide" refers to a polymer of amino acids and does not refer to a specific length of the product; thus, peptides, oligopeptides, and proteins are included within the definition of polypeptide. This term also does not refer to or exclude post-expression modifications of the polypeptide, for example, glycosylations, acetylations, phosphorylations and the like. Included within the definition are, for example, polypeptides containing one or more analogs of an amino acid (including, for example, unnatural amino acids, etc.), polypeptides with substituted linkages, as well as other modifications known in the art, both naturally occurring and non-naturally occurring.

"Transformation", as used herein, refers to the insertion of an exogenous polynucleotide into a host cell, irrespective of the method used for the insertion, for example, direct uptake, transduction, f-mating or electroporation. The exogenous polynucleotide may be maintained as a non-integrated vector, for example, a plasmid, or alternatively, may be integrated into the host genome.

"Treatment" as used herein refers to prophylaxis and/or therapy.

An "individual", as used herein, refers to vertebrates, particularly members of the mammalian species, and includes but is not limited to domestic animals, sports animals, and primates, including humans.

As used herein, the "sense strand" of a nucleic acid contains the sequence that has sequence homology to that of mRNA. The "anti-sense strand" contains a sequence which is complementary to that of the "sense strand".

As used herein, a "positive stranded genome" of a virus is one in which the genome, whether RNA or DNA, is single-stranded and which encodes a viral polypeptide(s). Examples of positive stranded RNA viruses include Togaviridae, Coronaviridae, Retroviridae, Picornaviridae, and Caliciviridae. Included also, are the Flaviviridae, which were formerly classified as Togaviridae. See Fields & Knipe (1986).

As used herein, "antibody-containing body component" refers to a component of an individual's body which is a source of the antibodies of interest. Antibody containing body components are known in the art, and include but are not limited to, for example, plasma, serum, spinal fluid, lymph fluid, the external sections of the respiratory, intestinal, and genitourinary tracts, tears, saliva, milk, white blood cells, and myelomas.

As used herein, "purified HCV" refers to a preparation of HCV which has been isolated from the cellular constituents with which the virus is normally associated, and from other types of viruses which may be present in the infected tissue. The techniques for isolating viruses are known to those of skill in the art, and include, for example, centrifugation and affinity chromatography; a method of preparing purified HCV is discussed *infra*.

The term "HCV particles" as used herein include entire virion as well as particles which are intermediates in virion formation. HCV particles generally have one or more HCV proteins associated with the HCV nucleic acid.

As used herein, the term "probe" refers to a polynucleotide which forms a hybrid structure with a sequence in a target region, due to complementarity of at least one sequence in the probe with a sequence in the target region. The probe, however, does not contain a sequence complementary to sequence(s) used to prime the polymerase chain reaction.

As used herein, the term "target region" refers to a region of the nucleic acid which is to be amplified and/or detected.

As used herein, the term "viral RNA", which includes HCV RNA, refers to RNA from the viral genome, fragments thereof, transcripts thereof, and mutant sequences derived therefrom.

As used herein, a "biological sample" refers to a sample of tissue or fluid isolated from an individual, including but not limited to, for example, plasma, serum, spinal fluid, lymph fluid, the external sections of the skin, respiratory, intestinal, and genitourinary tracts, tears, saliva, milk, blood cells, tumors, organs, and also samples of in vitro cell culture constituents (including but not limited to conditioned medium resulting from the

growth of cells in cell culture medium, putatively virally infected cells, recombinant cells, and cell components).

5 II. Description of the Invention

The practice of the present invention will employ, unless otherwise indicated, conventional techniques of molecular biology, microbiology, recombinant DNA, and immunology, which are within the skill of the art. Such techniques are explained fully in the literature. See e.g., Maniatis, Fitsch & Sambrook, MOLECULAR CLONING; A LABORATORY MANUAL (1982); DNA CLONING, VOLUMES I AND II (D.N Glover ed. 1985); OLIGONUCLEOTIDE SYNTHESIS (M.J. Gait ed, 1984); NUCLEIC ACID HYBRIDIZATION (B.D. Hames & S.J. Higgins eds. 1984); TRANSCRIPTION AND TRANSLATION (B.D. Hames & S.J. Higgins eds. 1984); ANIMAL CELL CULTURE (R.I. Freshney ed. 1986); IMMOBILIZED CELLS AND ENZYMES (IRL Press, 1986); B. Perbal, A PRACTICAL GUIDE TO MOLECULAR CLONING (1984); the series, METHODS IN ENZYMOLOGY (Academic Press, Inc.); GENE TRANSFER VECTORS FOR MAMMALIAN CELLS (J.H. Miller and M.P. Calos eds. 1987, Cold Spring Harbor Laboratory), Methods in Enzymology Vol. 154 and Vol. 155 (Wu and Grossman, and Wu, eds., respectively), Mayer and Walker, eds. (1987), IMMUNOCHEMICAL METHODS IN CELL AND MOLECULAR BIOLOGY (Academic Press, London), Scopes, (1987), PROTEIN PURIFICATION: PRINCIPLES AND PRACTICE, Second Edition (Springer-Verlag, N.Y.), and HANDBOOK OF EXPERIMENTAL IMMUNOLOGY, VOLUMES I-IV (D.M. Weir and C. C. Blackwell eds 1986). All patents, patent applications, and publications mentioned herein, both supra and infra, are hereby incorporated herein by reference.

The useful materials and processes of the present invention are made possible by the provision of a family of nucleotide sequences isolated from cDNA libraries which contain HCV cDNA sequences. These cDNA libraries

ies were derived from nucleic acid sequences present in the plasma of an HCV-infected chimpanzee. The construction of one of these libraries, the "c" library (ATCC No. 40394), was reported in EPO Pub. No. 318,216. Several of the clones containing HCV cDNA reported herein were obtained from the "c" library. Although other clones reported herein were obtained from other HCV cDNA libraries, the presence of clones containing the sequences in the "c" library was confirmed. As discussed in EPO Pub. No. 318,216, the family of HCV cDNA sequences isolated from the "c" library are not of human or chimpanzee origin, and show no significant homology to sequences contained within the HBV genome.

The availability of the HCV cDNAs described herein permits the construction of polynucleotide probes which are reagents useful for detecting viral polynucleotides in biological samples, including donated blood. For example, from the sequences it is possible to synthesize DNA oligomers of about 8-10 nucleotides, or larger, which are useful as hybridization probes to detect the presence of HCV RNA in, for example, donated blood, sera of subjects suspected of harboring the virus, or cell culture systems in which the virus is replicating. In addition, the cDNA sequences also allow the design and production of HCV specific polypeptides which are useful as diagnostic reagents for the presence of antibodies raised during HCV infection. Antibodies to purified polypeptides derived from the cDNAs may also be used to detect viral antigens in biological samples, including, for example, donated blood samples, sera from patients with NANBH, and in tissue culture systems being used for HCV replication. Moreover, the immunogenic polypeptides disclosed herein, which are encoded in portions of the ORF of HCV cDNA shown in Fig. 17, are also useful for HCV

screening, diagnosis, and treatment, and for raising antibodies which are also useful for these purposes.

5 In addition, the novel cDNA sequences described herein enable further characterization of the HCV genome. Polynucleotide probes and primers derived from these sequences may be used to amplify sequences present in cDNA libraries, and/or to screen cDNA libraries for additional
10 overlapping cDNA sequences, which, in turn, may be used to obtain more overlapping sequences. As indicated infra. and in EPO Pub. No. 318,216, the genome of HCV appears to be RNA comprised primarily of a large open reading frame (ORF) which encodes a large polyprotein.

15 The HCV cDNA sequences provided herein, the polypeptides derived from these sequences, and the immunogenic polypeptides described herein, as well as antibodies directed against these polypeptides are also useful in the isolation and identification of the blood-borne NABV (BB-NANBV) agent(s). For example, antibodies
20 directed against HCV epitopes contained in polypeptides derived from the cDNAs may be used in processes based upon affinity chromatography to isolate the virus. Alternatively, the antibodies may be used to identify viral particles isolated by other techniques. The viral
25 antigens and the genomic material within the isolated viral particles may then be further characterized.

In addition to the above, the information provided infra allows the identification of additional HCV strains or isolates. The isolation and characterization
30 of the additional HCV strains or isolates may be accomplished by isolating the nucleic acids from body components which contain viral particles and/or viral RNA, creating cDNA libraries using polynucleotide probes based on the HCV cDNA probes described infra., screening the
35 libraries for clones containing HCV cDNA sequences described infra., and comparing the HCV cDNAs from the new

isolates with the cDNAs described infra. The polypeptides encoded therein, or in the viral genome, may be monitored for immunological cross-reactivity utilizing the
5 polypeptides and antibodies described supra. Strains or isolates which fit within the parameters of HCV, as described in the Definitions section, supra., are readily identifiable. Other methods for identifying HCV strains
10 will be obvious to those of skill in the art, based upon the information provided herein.

Isolation of the HCV cDNA Sequences

The novel HCV cDNA sequences described infra. extend the sequence of the cDNA to the HCV genome reported
15 in EPO Pub. No. 318,216. The sequences which are present in clones b114a, 18g, ag30a, CA205a, CA290a, CA216a, p114a, CA167b, CA156e, CA84a, and CA59a lie upstream of the reported sequence, and when compiled, yield
20 nucleotides nos. -319 to 1348 of the composite HCV cDNA sequence. (The negative number on a nucleotide indicates its distance upstream of the nucleotide which starts the putative initiator MET codon.) The sequences which are present in clones b5a and 16jh lie downstream of the
25 reported sequence, and yield nucleotides nos. 8659 to 8866 of the composite sequence. The composite HCV cDNA sequence which includes the sequences in the aforementioned clones, is shown in Fig. 17.

The novel HCV cDNAs described herein were isolated from a number of HCV cDNA libraries, including
30 the "c" library present in lambda gt11 (ATCC No. 40394). The HCV cDNA libraries were constructed using pooled serum from a chimpanzee with chronic HCV infection and containing a high titer of the virus, i.e., at least 10^6 chimp infectious doses/ml (CID/ml). The pooled serum was used
35 to isolate viral particles; nucleic acids isolated from these particles was used as the template in the construc-

tion of cDNA libraries to the viral genome. The procedures for isolation of putative HCV particles and for constructing the "c" HCV cDNA library is described in EPO
5 Pub. No. 318,216. Other methods for constructing HCV cDNA libraries are known in the art, and some of these methods are described infra., in the Examples. Isolation of the sequences was by screening the libraries using synthetic
10 polynucleotide probes, the sequences of which were derived from the 5'-region and the 3'-region of the known HCV cDNA sequence. The description of the method to retrieve the cDNA sequences is mostly of historical interest. The resultant sequences (and their complements) are provided herein, and the sequences, or any portion thereof, could
15 be prepared using synthetic methods, or by a combination of synthetic methods with retrieval of partial sequences using methods similar to those described herein.

20 Preparation of Viral Polypeptides and Fragments

The availability of HCV cDNA sequences, or nucleotide sequences derived therefrom (including segments and modifications of the sequence), permits the construction of expression vectors encoding antigenically active regions of the polypeptide encoded in either strand.
25 These antigenically active regions may be derived from coat or envelope antigens or from core antigens, or from antigens which are non-structural including, for example, polynucleotide binding proteins, polynucleotide
30 polymerase(s), and other viral proteins required for the replication and/or assembly of the virus particle. Fragments encoding the desired polypeptides are derived from the cDNA clones using conventional restriction digestion or by synthetic methods, and are ligated into vectors which may, for example, contain portions of fusion
35 sequences such as beta-galactosidase or superoxide dismutase (SOD), preferably SOD. Methods and vectors

which are useful for the production of polypeptides which contain fusion sequences of SOD are described in European Patent Office Publication number 0196056, published
5 October 1, 1986. Vectors for the expression of fusion polypeptides of SOD and HCV polypeptides encoded in a number of HCV clones are described infra., in the Examples. Any desired portion of the HCV cDNA containing an open reading frame, in either sense strand, can be
10 obtained as a recombinant polypeptide, such as a mature or fusion protein; alternatively, a polypeptide encoded in the cDNA can be provided by chemical synthesis.

The DNA encoding the desired polypeptide, whether in fused or mature form, and whether or not
15 containing a signal sequence to permit secretion, may be ligated into expression vectors suitable for any convenient host. Both eukaryotic and prokaryotic host systems are presently used in forming recombinant polypeptides, and a summary of some of the more common
20 control systems and host cell lines is given infra. The polypeptide is then isolated from lysed cells or from the culture medium and purified to the extent needed for its intended use. Purification may be by techniques known in the art, for example, differential extraction, salt
25 fractionation, chromatography on ion exchange resins, affinity chromatography, centrifugation, and the like. See, for example, Methods in Enzymology for a variety of methods for purifying proteins. Such polypeptides can be used as diagnostics, or those which give rise to neutral-
30 izing antibodies may be formulated into vaccines. Antibodies raised against these polypeptides can also be used as diagnostics, or for passive immunotherapy. In addition, as discussed infra., antibodies to these
35 polypeptides are useful for isolating and identifying HCV particles.

Preparation of Antigenic Polypeptides and Conjugation with Carrier

5 An antigenic region of a polypeptide is generally relatively small--typically 8 to 10 amino acids or less in length. Fragments of as few as 5 amino acids may characterize an antigenic region. These segments may correspond to regions of HCV antigen. Accordingly, using the
10 cDNAs of HCV as a basis, DNAs encoding short segments of HCV polypeptides can be expressed recombinantly either as fusion proteins, or as isolated polypeptides. In addition, short amino acid sequences can be conveniently obtained by chemical synthesis. In instances wherein the
15 synthesized polypeptide is correctly configured so as to provide the correct epitope, but is too small to be immunogenic, the polypeptide may be linked to a suitable carrier.

 A number of techniques for obtaining such linkage are known in the art, including the formation of
20 disulfide linkages using N-succinimidyl-3-(2-pyridylthio)propionate (SPDP) and succinimidyl 4-(N-maleimido-methyl)cyclohexane-1-carboxylate (SMCC) obtained from Pierce Company, Rockford, Illinois, (if the peptide lacks a sulfhydryl group, this can be provided by addition of a
25 cysteine residue.) These reagents create a disulfide linkage between themselves and peptide cysteine residues on one protein and an amide linkage through the epsilon-amino on a lysine, or other free amino group in the other. A variety of such disulfide/amide-forming agents are
30 known. See, for example, Immun. Rev. (1982) 62:185. Other bifunctional coupling agents form a thioether rather than a disulfide linkage. Many of these thio-ether-forming agents are commercially available and include reactive esters of 6-maleimidocaproic acid, 2-bromoacetic
35 acid, 2-iodoacetic acid, 4-(N-maleimido-methyl)cyclohexane-1-carboxylic acid, and the like. The

carboxyl groups can be activated by combining them with succinimide or 1-hydroxyl-2-nitro-4-sulfonic acid, sodium salt. Additional methods of coupling antigens employs the rotavirus/"binding peptide" system described in EPO Pub. No. 259,149, the disclosure of which is incorporated herein by reference. The foregoing list is not meant to be exhaustive, and modifications of the named compounds can clearly be used.

Any carrier may be used which does not itself induce the production of antibodies harmful to the host. Suitable carriers are typically large, slowly metabolized macromolecules such as proteins; polysaccharides, such as latex functionalized sepharose, agarose, cellulose, cellulose beads and the like; polymeric amino acids, such as polyglutamic acid, polylysine, and the like; amino acid copolymers; and inactive virus particles. Especially useful protein substrates are serum albumins, keyhole limpet hemocyanin, immunoglobulin molecules, thyroglobulin, ovalbumin, tetanus toxoid, and other proteins well known to those skilled in the art.

In addition to full-length viral proteins, polypeptides comprising truncated HCV amino acid sequences encoding at least one viral epitope are useful immunological reagents. For example, polypeptides comprising such truncated sequences can be used as reagents in an immunoassay. These polypeptides also are candidate subunit antigens in compositions for antiserum production or vaccines. While these truncated sequences can be produced by various known treatments of native viral protein, it is generally preferred to make synthetic or recombinant polypeptides comprising an HCV sequence. Polypeptides comprising these truncated HCV sequences can be made up entirely of HCV sequences (one or more epitopes, either contiguous or noncontiguous), or HCV sequences and heterologous sequences in a fusion protein.

Useful heterologous sequences include sequences that provide for secretion from a recombinant host, enhance the immunological reactivity of the HCV epitope(s), or facilitate the coupling of the polypeptide to an immunoassay support or a vaccine carrier. See, e.g., EPO Pub. No. 116,201; U.S. Pat. No. 4,722,840; EPO Pub. No. 259,149; U.S. Pat. No. 4,629,783, the disclosures of which are incorporated herein by reference.

The size of polypeptides comprising the truncated HCV sequences can vary widely, the minimum size being a sequence of sufficient size to provide an HCV epitope, while the maximum size is not critical. For convenience, the maximum size usually is not substantially greater than that required to provide the desired HCV epitopes and function(s) of the heterologous sequence, if any. Typically, the truncated HCV amino acid sequence will range from about 5 to about 100 amino acids in length. More typically, however, the HCV sequence will be a maximum of about 50 amino acids in length, preferably a maximum of about 30 amino acids. It is usually desirable to select HCV sequences of at least about 10, 12 or 15 amino acids, up to a maximum of about 20 or 25 amino acids.

Truncated HCV amino acid sequences comprising epitopes can be identified in a number of ways. For example, the entire viral protein sequence can be screened by preparing a series of short peptides that together span the entire protein sequence. An example of antigenic screening of the regions of the HCV polyprotein is shown infra. In addition, by starting with, for example, 100mer polypeptides, it would be routine to test each polypeptide for the presence of epitope(s) showing a desired reactivity, and then testing progressively smaller and overlapping fragments from an identified 100mer to map the epitope of interest. Screening such peptides in an

immunoassay is within the skill of the art. It is also known to carry out a computer analysis of a protein sequence to identify potential epitopes, and then prepare oligopeptides comprising the identified regions for screening. Such a computer analysis of the HCV amino acid sequence is shown in Fig. 20, where the hydrophilic/hydrophobic character is displayed above the antigen index. The amino acids are numbered from the starting MET (position 1) as shown in Fig. 17. It is appreciated by those of skill in the art that such computer analysis of antigenicity does not always identify an epitope that actually exists, and can also incorrectly identify a region of the protein as containing an epitope.

Examples of HCV amino acid sequences that may be useful, which are expressed from expression vectors comprised of clones 5-1-1, 81, CA74a, 35f, 279a, C36, C33b, CA290a, C8f, C12f, 14c, 15e, C25c, C33c, C33f, 33g, C39c, C40b, CA167b are described infra. Other examples of HCV amino acid sequences that may be useful as described herein are set forth below. It is to be understood that these peptides do not necessarily precisely map one epitope, and may also contain HCV sequence that is not immunogenic. These non-immunogenic portions of the sequence can be defined as described above using conventional techniques and deleted from the described sequences. Further, additional truncated HCV amino acid sequences that comprise an epitope or are immunogenic can be identified as described above. The following sequences are given by amino acid number (i.e., "AA n ") where n is the amino acid number as shown in Fig. 17:

AA1-AA25; AA1-AA50; AA1-AA84; AA9-AA177; AA1-AA10;
AA5-AA20; AA20-AA25; AA35-AA45; AA50-AA100;
AA40-AA90; AA45-AA65; AA65-AA75; AA80-90; AA99-AA120;
AA95-AA110; AA105-AA120; AA100-AA150; AA150-AA200;
AA155-AA170; AA190-AA210; AA200-AA250; AA220-AA240;

5 AA245-AA265; AA250-AA300; AA290-AA330; AA290-305;
AA300-AA350; AA310-AA330; AA350-AA400; AA380-AA395;
AA405-AA495; AA400-AA450; AA405-AA415; AA415-AA425;
AA425-AA435; AA437-AA582; AA450-AA500; AA440-AA460;
AA460-AA470; AA475-AA495; AA500-AA550; AA511-AA690;
AA515-AA550; AA550-AA600; AA550-AA625; AA575-AA605;
AA585-AA600; AA600-AA650; AA600-AA625; AA635-AA665;
AA650-AA700; AA645-AA680; AA700-AA750; AA700-AA725;
AA700-AA750; AA725-AA775; AA770-AA790; AA750-AA800;
AA800-AA815; AA825-AA850; AA850-AA875; AA800-AA850;
AA920-AA990; AA850-AA900; AA920-AA945; AA940-AA965;
10 AA970-AA990; AA950-AA1000; AA1000-AA1060;
AA1000-AA1025; AA1000-AA1050; AA1025-AA1040;
AA1040-AA1055; AA1075-AA1175; AA1050-AA1200;
AA1070-AA1100; AA1100-AA1130; AA1140-AA1165;
AA1192-AA1457; AA1195-AA1250; AA1200-AA1225;
AA1225-AA1250; AA1250-AA1300; AA1260-AA1310;
AA1260-AA1280; AA1266-AA1428; AA1300-AA1350;
15 AA1290-AA1310; AA1310-AA1340; AA1345-AA1405;
AA1345-AA1365; AA1350-AA1400; AA1365-AA1380;
AA1380-AA1405; AA1400-AA1450; AA1450-AA1500;
AA1460-AA1475; AA1475-AA1515; AA1475-AA1500;
AA1500-AA1550; AA1500-AA1515; AA1515-AA1550;
AA1550-AA1600; AA1545-AA1560; AA1569-AA1931;
AA1570-AA1590; AA1595-AA1610; AA1590-AA1650;
AA1610-AA1645; AA1650-AA1690; AA1685-AA1770;
20 AA1689-AA1805; AA1690-AA1720; AA1694-AA1735;
AA1720-AA1745; AA1745-AA1770; AA1750-AA1800;
AA1775-AA1810; AA1795-AA1850; AA1850-AA1900;
AA1900-AA1950; AA1900-AA1920; AA1916-AA2021;
AA1920-AA1940; AA1949-AA2124; AA1950-AA2000;
AA1950-AA1985; AA1980-AA2000; AA2000-AA2050;
AA2005-AA2025; AA2020-AA2045; AA2045-AA2100;
25 AA2045-AA2070; AA2054-AA2223; AA2070-AA2100;
AA2100-AA2150; AA2150-AA2200; AA2200-AA2250;
AA2200-AA2325; AA2250-AA2330; AA2255-AA2270;
AA2265-AA2280; AA2280-AA2290; AA2287-AA2385;
AA2300-AA2350; AA2290-AA2310; AA2310-AA2330;
AA2330-AA2350; AA2350-AA2400; AA2348-AA2464;
AA2345-AA2415; AA2345-AA2375; AA2370-AA2410;
AA2371-AA2502; AA2400-AA2450; AA2400-AA2425;
30 AA2415-AA2450; AA2445-AA2500; AA2445-AA2475;
AA2470-AA2490; AA2500-AA2550; AA2505-AA2540;
AA2535-AA2560; AA2550-AA2600; AA2560-AA2580;
AA2600-AA2650; AA2605-AA2620; AA2620-AA2650;
AA2640-AA2660; AA2650-AA2700; AA2655-AA2670;
AA2670-AA2700; AA2700-AA2750; AA2740-AA2760;
AA2750-AA2800; AA2755-AA2780;
35 AA2780-AA2830; AA2785-AA2810; AA2796-AA2886;
AA2810-AA2825; AA2800-AA2850; AA2850-AA2900;
AA2850-AA2865; AA2885-AA2905; AA2900-AA2950;

AA2910-AA2930; AA2925-AA2950; AA2945-end(C' terminal).

5 The above HCV amino acid sequences can be prepared as discrete peptides or incorporated into a larger polypeptide, and may find use as described herein. Additional polypeptides comprising truncated HCV sequences are described in the examples.

10 The observed relationship of the putative polyproteins of HCV and the Flaviviruses allows some prediction of the putative domains of the HCV "non-structural" (NS) proteins. The locations of the individual NS proteins in the putative Flavivirus precursor polyprotein are fairly well-known. Moreover, these
15 also coincide with observed gross fluctuations in the hydrophobicity profile of the polyprotein. It is established that NS5 of Flaviviruses encodes the virion polymerase, and that NS1 corresponds with a complement fixation antigen which has been shown to be an effective
20 vaccine in animals. Recently, it has been shown that a flaviviral protease function resides in NS3. Due to the observed similarities between HCV and the Flaviviruses, described infra., deductions concerning the approximate locations of the corresponding protein domains and func-
25 tions in the HCV polyprotein are possible. The expression of polypeptides containing these domains in a variety of recombinant host cells, including, for example, bacteria, yeast, insect, and vertebrate cells, should give rise to important immunological reagents which can be used for
30 diagnosis, detection, and vaccines.

Although the non-structural protein regions of the putative polyproteins of the HCV isolate described herein and of Flaviviruses appear to have some similarity,
35 there is less similarity between the putative structural regions which are towards the N-terminus. In this region, there is a greater divergence in sequence, and in addi-

tion, the hydrophobic profile of the two regions show less similarity. This "divergence" begins in the N-terminal region of the putative NS1 domain in HCV, and extends to the presumed N-terminus. Nevertheless, it may still be possible to predict the approximate locations of the putative nucleocapsid (N-terminal basic domain) and E (generally hydrophobic) domains within the HCV polyprotein. In the Examples the predictions are based on the changes observed in the hydrophobic profile of the HCV polyprotein, and on a knowledge of the location and character of the flaviviral proteins. From these predictions it may be possible to identify approximate regions of the HCV polyprotein that could correspond with useful immunological reagents. For example, the E and NS1 proteins of Flaviviruses are known to have efficacy as protective vaccines. These regions, as well as some which are shown to be antigenic in the HCV isolate described herein, for example those within putative NS3, C, and NS5, etc., should also provide diagnostic reagents. Moreover, the location and expression of viral-encoded enzymes may also allow the evaluation of anti-viral enzyme inhibitors, i.e., for example, inhibitors which prevent enzyme activity by virtue of an interaction with the enzyme itself, or substances which may prevent expression of the enzyme, (for example, anti-sense RNA, or other drugs which interfere with expression).

Preparation of Hybrid Particle Immunogens Containing HCV Epitopes

The immunogenicity of the epitopes of HCV may also be enhanced by preparing them in mammalian or yeast systems fused with or assembled with particle-forming proteins such as, for example, that associated with hepatitis B surface antigen. Constructs wherein the NANBV epitope is linked directly to the particle-forming protein

coding sequences produce hybrids which are immunogenic with respect to the HCV epitope. In addition, all of the vectors prepared include epitopes specific to HBV, having various degrees of immunogenicity, such as, for example, the pre-S peptide. Thus, particles constructed from particle forming protein which include HCV sequences are immunogenic with respect to HCV and HBV.

Hepatitis surface antigen (HBSAg) has been shown to be formed and assembled into particles in S. cerevisiae (Valenzuela et al. (1982)), as well as in, for example, mammalian cells (Valenzuela, P., et al. (1984)). The formation of such particles has been shown to enhance the immunogenicity of the monomer subunit. The constructs may also include the immunodominant epitope of HBSAg, comprising the 55 amino acids of the presurface (pre-S) region. Neurath et al. (1984). Constructs of the pre-S-HBSAg particle expressible in yeast are disclosed in EPO 174,444, published March 19, 1986; hybrids including heterologous viral sequences for yeast expression are disclosed in EPO 175,261, published March 26, 1986. These constructs may also be expressed in mammalian cells such as Chinese hamster ovary (CHO) cells using an SV40-dihydrofolate reductase vector (Michelle et al. (1984)).

In addition, portions of the particle-forming protein coding sequence may be replaced with codons encoding an HCV epitope. In this replacement, regions which are not required to mediate the aggregation of the units to form immunogenic particles in yeast or mammals can be deleted, thus eliminating additional HBV antigenic sites from competition with the HCV epitope.

Preparation of Vaccines

Vaccines may be prepared from one or more immunogenic polypeptides derived from HCV cDNA, including the cDNA sequences described in the Examples. The

observed homology between HCV and Flaviviruses provides information concerning the polypeptides which may be most effective as vaccines, as well as the regions of the genome in which they are encoded. The general structure of the Flavivirus genome is discussed in Rice et al (1986). The flavivirus genomic RNA is believed to be the only virus-specific mRNA species, and it is translated into the three viral structural proteins, i.e., C, M, and E, as well as two large nonstructural proteins, NS4 and NS5, and a complex set of smaller nonstructural proteins. It is known that major neutralizing epitopes for Flaviviruses reside in the E (envelope) protein (Roehrig (1986)). Thus, vaccines may be comprised of recombinant polypeptides containing epitopes of HCV E. These polypeptides may be expressed in bacteria, yeast, or mammalian cells, or alternatively may be isolated from viral preparations. It is also anticipated that the other structural proteins may also contain epitopes which give rise to protective anti-HCV antibodies. Thus, polypeptides containing the epitopes of E, C, and M may also be used, whether singly or in combination, in HCV vaccines.

In addition to the above, it has been shown that immunization with NS1 (nonstructural protein 1), results in protection against yellow fever (Schlesinger et al (1986)). This is true even though the immunization does not give rise to neutralizing antibodies. Thus, particularly since this protein appears to be highly conserved among Flaviviruses, it is likely that HCV NS1 will also be protective against HCV infection. Moreover, it also shows that nonstructural proteins may provide protection against viral pathogenicity, even if they do not cause the production of neutralizing antibodies.

The information provided in the Examples concerning the immunogenicity of the polypeptides

expressed from cloned HCV cDNAs which span the various regions of the HCV ORF also allows predictions concerning their use in vaccines.

5 In view of the above, multivalent vaccines against HCV may be comprised of one or more epitopes from one or more structural proteins, and/or one or more epitopes from one or more nonstructural proteins. These vaccines may be comprised of, for example, recombinant HCV
10 polypeptides and/or polypeptides isolated from the virions. In particular, vaccines are contemplated comprising one or more of the following HCV proteins, or subunit antigens derived therefrom: E, NS1, C, NS2, NS3, NS4 and NS5. Particularly preferred are vaccines compris-
15 ing E and/or NS1, or subunits thereof.

The preparation of vaccines which contain an immunogenic polypeptide(s) as active ingredients, is known to one skilled in the art. Typically, such vaccines are prepared as injectables, either as liquid solutions or
20 suspensions; solid forms suitable for solution in, or suspension in, liquid prior to injection may also be prepared. The preparation may also be emulsified, or the protein encapsulated in liposomes. The active immunogenic ingredients are often mixed with excipients which are
25 pharmaceutically acceptable and compatible with the active ingredient. Suitable excipients are, for example, water, saline, dextrose, glycerol, ethanol, or the like and combinations thereof. In addition, if desired, the vac-
30 cine may contain minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents, and/or adjuvants which enhance the effectiveness of the vaccine. Examples of adjuvants which may be effec-
35 tive include but are not limited to: aluminum hydroxide, N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP), N-acetyl-nor-muramyl-L-alanyl-D-isoglutamine (CGP 11637, referred to as nor-MDP), N-acetylmuramyl-L-alanyl-D-

isoglutaminyl-L-alanine-2-(1'-2'-dipalmitoyl-sn-glycero-3-hydroxyphosphoryloxy)-ethylamine (CGP 19835A, referred to as MTP-PE), and RIBI, which contains three components
5 extracted from bacteria, monophosphoryl lipid A, trehalose dimycolate and cell wall skeleton (MPL+TDM+CWS) in a 2% squalene/Tween 80 emulsion. The effectiveness of an adjuvant may be determined by measuring the amount of
10 antibodies directed against an immunogenic polypeptide containing an HCV antigenic sequence resulting from administration of this polypeptide in vaccines which are also comprised of the various adjuvants.

The vaccines are conventionally administered
15 parenterally, by injection, for example, either subcutaneously or intramuscularly. Additional formulations which are suitable for other modes of administration include suppositories and, in some cases, oral formulations. For
20 suppositories, traditional binders and carriers may include, for example, polyalkylene glycols or triglycerides; such suppositories may be formed from mixtures containing the active ingredient in the range of 0.5% to 10%, preferably 1%-2%. Oral formulations include
25 such normally employed excipients as, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, and the like. These compositions
30 take the form of solutions, suspensions, tablets, pills, capsules, sustained release formulations or powders and contain 10%-95% of active ingredient, preferably 25%-70%.

The proteins may be formulated into the vaccine as neutral or salt forms. Pharmaceutically acceptable
35 salts include the acid addition salts (formed with free amino groups of the peptide) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids such as acetic, oxalic, tartaric, maleic, and the like. Salts formed with

the free carboxyl groups may also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic
5 bases as isopropylamine, trimethylamine, 2-ethylamino ethanol, histidine, procaine, and the like.

Dosage and Administration of Vaccines

10 The vaccines are administered in a manner compatible with the dosage formulation, and in such amount as will be prophylactically and/or therapeutically effective. The quantity to be administered, which is generally
15 in the range of 5 micrograms to 250 micrograms of antigen per dose, depends on the subject to be treated, capacity of the subject's immune system to synthesize antibodies, and the degree of protection desired. Precise amounts of active ingredient required to be administered may depend
20 on the judgment of the practitioner and may be peculiar to each subject.

The vaccine may be given in a single dose schedule, or preferably in a multiple dose schedule. A
25 multiple dose schedule is one in which a primary course of vaccination may be with 1-10 separate doses, followed by other doses given at subsequent time intervals required to maintain and or reenforce the immune response, for
30 example, at 1-4 months for a second dose, and if needed, a subsequent dose(s) after several months. The dosage regimen will also, at least in part, be determined by the need of the individual and be dependent upon the judgment
of the practitioner.

In addition, the vaccine containing the immunogenic HCV antigen(s) may be administered in conjunction with other immunoregulatory agents, for example, im-
35 mune globulins.

Preparation of Antibodies Against HCV Epitopes

The immunogenic polypeptides prepared as described above are used to produce antibodies, both polyclonal and monoclonal. If polyclonal antibodies are desired, a selected mammal (e.g., mouse, rabbit, goat, horse, etc.) is immunized with an immunogenic polypeptide bearing an HCV epitope(s). Serum from the immunized animal is collected and treated according to known procedures. If serum containing polyclonal antibodies to an HCV epitope contains antibodies to other antigens, the polyclonal antibodies can be purified by immunoaffinity chromatography. Techniques for producing and processing polyclonal antisera are known in the art, see for example, Mayer and Walker (1987).

Alternatively, polyclonal antibodies may be isolated from a mammal which has been previously infected with HCV. An example of a method for purifying antibodies to HCV epitopes from serum from an infected individual, based upon affinity chromatography and utilizing a fusion polypeptide of SOD and a polypeptide encoded within cDNA clone 5-1-1, is presented in EPO Pub. No. 318,216.

Monoclonal antibodies directed against HCV epitopes can also be readily produced by one skilled in the art. The general methodology for making monoclonal antibodies by hybridomas is well known. Immortal antibody-producing cell lines can be created by cell fusion, and also by other techniques such as direct transformation of B lymphocytes with oncogenic DNA, or transfection with Epstein-Barr virus. See, e.g., M. Schreier et al. (1980); Hammerling et al. (1981); Kennett et al. (1980); see also, U.S. Patent Nos. 4,341,761; 4,399,121; 4,427,783; 4,444,887; 4,466,917; 4,472,500; 4,491,632; and 4,493,890. Panels of monoclonal antibodies produced against HCV epitopes can be screened for various properties; i.e., for isotype, epitope affinity, etc.

Antibodies, both monoclonal and polyclonal, which are directed against HCV epitopes are particularly useful in diagnosis, and those which are neutralizing are useful in passive immunotherapy. Monoclonal antibodies, in particular, may be used to raise anti-idiotypic antibodies.

Anti-idiotypic antibodies are immunoglobulins which carry an "internal image" of the antigen of the infectious agent against which protection is desired. See, for example, Nisonoff, A., et al. (1981) and Dreesman et al. (1985).

Techniques for raising anti-idiotypic antibodies are known in the art. See, for example, Grzych (1985), MacNamara et al. (1984), and Uytdehaag et al. (1985). These anti-idiotypic antibodies may also be useful for treatment and/or diagnosis of NANBH, as well as for an elucidation of the immunogenic regions of HCV antigens.

It would also be recognized by one of ordinary skill in the art that a variety of types of antibodies directed against HCV epitopes may be produced. As used herein, the term "antibody" refers to a polypeptide or group of polypeptides which are comprised of at least one antibody combining site. An "antibody combining site" or "binding domain" is formed from the folding of variable domains of an antibody molecule(s) to form three-dimensional binding spaces with an internal surface shape and charge distribution complementary to the features of an epitope of an antigen, which allows an immunological reaction with the antigen. An antibody combining site may be formed from a heavy and/or a light chain domain (VH and VL, respectively), which form hypervariable loops which contribute to antigen binding. The term "antibody" includes, for example, vertebrate antibodies, hybrid antibodies, chimeric antibodies, altered antibodies, univalent

antibodies, the Fab proteins, and single domain antibodies.

5 A "single domain antibody" (dAb) is an antibody which is comprised of an VH domain, which reacts immunologically with a designated antigen. A dAb does not contain a VL domain, but may contain other antigen binding domains known to exist in antibodies, for example, the kappa and lambda domains. Methods for preparing dAbs are known in the art. See, for example, Ward et al. (1989).

10 Antibodies may also be comprised of VH and VL domains, as well as other known antigen binding domains. Examples of these types of antibodies and methods for their preparation are known in the art (see, e.g., U.S. Patent No. 4,816,467, which is incorporated herein by reference), and include the following. For example, "vertebrate antibodies" refers to antibodies which are tetramers or aggregates thereof, comprising light and heavy chains which are usually aggregated in a "Y" configuration and which may or may not have covalent linkages between the chains. In vertebrate antibodies, the amino acid sequences of all the chains of a particular antibody are homologous with the chains found in one antibody produced by the lymphocyte which produces that antibody in situ, or in vitro (for example, in hybridomas).
15 Vertebrate antibodies typically include native antibodies, for example, purified polyclonal antibodies and monoclonal antibodies. Examples of the methods for the preparation of these antibodies are described infra.

20 "Hybrid antibodies" are antibodies wherein one pair of heavy and light chains is homologous to those in a first antibody, while the other pair of heavy and light chains is homologous to those in a different second antibody. Typically, each of these two pairs will bind different epitopes, particularly on different antigens. This results in the property of "divalence", i.e., the ability
25
30
35

to bind two antigens simultaneously. Such hybrids may also be formed using chimeric chains, as set forth below.

5 "Chimeric antibodies", are antibodies in which the heavy and/or light chains are fusion proteins. Typically the constant domain of the chains is from one particular species and/or class, and the variable domains are from a different species and/or class. Also included is any antibody in which either or both of the heavy or
10 light chains are composed of combinations of sequences mimicking the sequences in antibodies of different sources, whether these sources be differing classes, or different species of origin, and whether or not the fusion point is at the variable/constant boundary. Thus, it is
15 possible to produce antibodies in which neither the constant nor the variable region mimic known antibody sequences. It then becomes possible, for example, to construct antibodies whose variable region has a higher specific affinity for a particular antigen, or whose
20 constant region can elicit enhanced complement fixation, or to make other improvements in properties possessed by a particular constant region.

Another example is "altered antibodies", which refers to antibodies in which the naturally occurring
25 amino acid sequence in a vertebrate antibody has been varied. Utilizing recombinant DNA techniques, antibodies can be redesigned to obtain desired characteristics. The possible variations are many, and range from the changing of one or more amino acids to the complete redesign of a
30 region, for example, the constant region. Changes in the constant region, in general, to attain desired cellular process characteristics, e.g., changes in complement fixation, interaction with membranes, and other effector functions. Changes in the variable region may be made to
35 alter antigen binding characteristics. The antibody may also be engineered to aid the specific delivery of a

molecule or substance to a specific cell or tissue site. The desired alterations may be made by known techniques in molecular biology, e.g., recombinant techniques, site
5 directed mutagenesis, etc.

Yet another example are "univalent antibodies", which are aggregates comprised of a heavy chain/light chain dimer bound to the Fc (i.e., constant) region of a second heavy chain. This type of antibody escapes
10 antigenic modulation. See, e.g., Glennie et al. (1982).

Included also within the definition of antibodies are "Fab" fragments of antibodies. The "Fab" region refers to those portions of the heavy and light chains which are roughly equivalent, or analogous, to the
15 sequences which comprise the branch portion of the heavy and light chains, and which have been shown to exhibit immunological binding to a specified antigen, but which lack the effector Fc portion. "Fab" includes aggregates
20 of one heavy and one light chain (commonly known as Fab'), as well as tetramers containing the 2H and 2L chains (referred to as $F(ab)_2$), which are capable of selectively reacting with a designated antigen or antigen family. "Fab" antibodies may be divided into subsets analogous to those described above, i.e., "vertebrate Fab", "hybrid
25 Fab", "chimeric Fab", and "altered Fab". Methods of producing "Fab" fragments of antibodies are known within the art and include, for example, proteolysis, and synthesis by recombinant techniques.

30 II.H. Diagnostic Oligonucleotide Probes and Kits

Using the disclosed portions of the isolated HCV cDNAs as a basis, oligomers of approximately 8 nucleotides or more can be prepared, either by excision or
35 synthetically, which hybridize with the HCV genome and are useful in identification of the viral agent(s), further characterization of the viral genome(s), as well as in

5 detection of the virus(es) in diseased individuals. The
probes for HCV polynucleotides (natural or derived) are a
length which allows the detection of unique viral
10 sequences by hybridization. While 6-8 nucleotides may be
a workable length, sequences of 10-12 nucleotides are
preferred, and about 20 nucleotides appears optimal.
Preferably, these sequences will derive from regions which
lack heterogeneity. These probes can be prepared using
15 routine methods, including automated oligonucleotide
synthetic methods. Among useful probes, for example, are
those derived from the newly isolated clones disclosed
herein, as well as the various oligomers useful in probing
cDNA libraries, set forth below. A complement to any
20 unique portion of the HCV genome will be satisfactory.
For use as probes, complete complementarity is desirable,
though it may be unnecessary as the length of the fragment
is increased.

20 For use of such probes as diagnostics, the bio-
logical sample to be analyzed, such as blood or serum, may
be treated, if desired, to extract the nucleic acids
contained therein. The resulting nucleic acid from the
sample may be subjected to gel electrophoresis or other
25 size separation techniques; alternatively, the nucleic
acid sample may be dot blotted without size separation.
The probes are then labeled. Suitable labels, and methods
for labeling probes are known in the art, and include, for
example, radioactive labels incorporated by nick transla-
30 tion or kinasing, biotin, fluorescent probes, and
chemiluminescent probes. The nucleic acids extracted from
the sample are then treated with the labeled probe under
hybridization conditions of suitable stringencies, and
polynucleotide duplexes containing the probe are detected.

35 The probes can be made completely complementary
to the HCV genome. Therefore, usually high stringency
conditions are desirable in order to prevent false

positives. However, conditions of high stringency should only be used if the probes are complementary to regions of the viral genome which lack heterogeneity. The stringency of hybridization is determined by a number of factors during hybridization and during the washing procedure, including temperature, ionic strength, length of time, and concentration of formamide. These factors are outlined in, for example, Maniatis, T. (1982).

Generally, it is expected that the HCV genome sequences will be present in serum of infected individuals at relatively low levels, i.e., at approximately 10^2 - 10^3 chimp infectious doses (CID) per ml. This level may require that amplification techniques be used in hybridization assays. Such techniques are known in the art. For example, the Enzo Biochemical Corporation "Bio-Bridge" system uses terminal deoxynucleotide transferase to add unmodified 3'-poly-dT-tails to a DNA probe. The poly dT-tailed probe is hybridized to the target nucleotide sequence, and then to a biotin-modified poly-A. PCT application 84/03520 and EPA124221 describe a DNA hybridization assay in which: (1) analyte is annealed to a single-stranded DNA probe that is complementary to an enzyme-labeled oligonucleotide; and (2) the resulting tailed duplex is hybridized to an enzyme-labeled oligonucleotide. EPA 204510 describes a DNA hybridization assay in which analyte DNA is contacted with a probe that has a tail, such as a poly-dT tail, an amplifier strand that has a sequence that hybridizes to the tail of the probe, such as a poly-A sequence, and which is capable of binding a plurality of labeled strands. A particularly desirable technique may first involve amplification of the target HCV sequences in sera approximately 10,000 fold, i.e., to approximately 10^6 sequences/ml. This may be accomplished, for example, by the polymerase chain reactions (PCR) technique described which is by Saiki et al. (1986),

by Mullis, U.S. Patent No. 4,683,195, and by Mullis et al. U.S. Patent No. 4,683,202. The amplified sequence(s) may then be detected using a hybridization assay which is described in EP 317,077, published May 24, 1989. These hybridization assays, which should detect sequences at the level of 10^6 /ml, utilize nucleic acid multimers which bind to single-stranded analyte nucleic acid, and which also bind to a multiplicity of single-stranded labeled oligonucleotides. A suitable solution phase sandwich assay which may be used with labeled polynucleotide probes, and the methods for the preparation of probes is described in EPO 225,807, published June 16, 1987.

The probes can be packaged into diagnostic kits. Diagnostic kits include the probe DNA, which may be labeled; alternatively, the probe DNA may be unlabeled and the ingredients for labeling may be included in the kit in separate containers. The kit may also contain other suitably packaged reagents and materials needed for the particular hybridization protocol, for example, standards, as well as instructions for conducting the test.

Immunoassay and Diagnostic Kits

Both the polypeptides which react immunologically with serum containing HCV antibodies, for example, those detected by the antigenic screening method described infra. in the Examples, as well those derived from or encoded within the isolated clones described in the Examples, and composites thereof, and the antibodies raised against the HCV specific epitopes in these polypeptides, are useful in immunoassays to detect presence of HCV antibodies, or the presence of the virus and/or viral antigens, in biological samples. Design of the immunoassays is subject to a great deal of variation, and a variety of these are known in the art. For example, the immunoassay may utilize one viral epitope; alternatively,

the immunoassay may use a combination of viral epitopes derived from these sources; these epitopes may be derived from the same or from different viral polypeptides, and
5 may be in separate recombinant or natural polypeptides, or together in the same recombinant polypeptides. It may use, for example, a monoclonal antibody directed towards a viral epitope(s), a combination of monoclonal antibodies directed towards epitopes of one viral antigen, monoclonal
10 antibodies directed towards epitopes of different viral antigens, polyclonal antibodies directed towards the same viral antigen, or polyclonal antibodies directed towards different viral antigens. Protocols may be based, for example, upon competition, or direct reaction, or sandwich
15 type assays. Protocols may also, for example, use solid supports, or may be by immunoprecipitation. Most assays involve the use of labeled antibody or polypeptide; the labels may be, for example, fluorescent, chemiluminescent, radioactive, or dye molecules. Assays which amplify the
20 signals from the probe are also known; examples of which are assays which utilize biotin and avidin, and enzyme-labeled and mediated immunoassays, such as ELISA assays.

Some of the antigenic regions of the putative polyprotein have been mapped and identified by screening
25 the antigenicity of bacterial expression products of HCV cDNAs which encode portions of the polyprotein. See the Examples. Other antigenic regions of HCV may be detected by expressing the portions of the HCV cDNAs in other
30 expression systems, including yeast systems and cellular systems derived from insects and vertebrates. In addition, studies giving rise to an antigenicity index and hydrophobicity/hydrophilicity profile give rise to information concerning the probability of a region's
35 antigenicity.

The studies on antigenic mapping by expression of HCV cDNAs showed that a number of clones containing

these cDNAs expressed polypeptides which were immunologically reactive with serum from individuals with NANBH. No single polypeptide was immunologically reactive
5 with all sera. Five of these polypeptides were very immunogenic in that antibodies to the HCV epitopes in these polypeptides were detected in many different patient sera, although the overlap in detection was not complete. Thus, the results on the immunogenicity of the polypeptides
10 encoded in the various clones suggest that efficient detection systems may include the use of panels of epitopes. The epitopes in the panel may be constructed into one or multiple polypeptides.

Kits suitable for immunodiagnosis and containing the appropriate labeled reagents are constructed by
15 packaging the appropriate materials, including the polypeptides of the invention containing HCV epitopes or antibodies directed against HCV epitopes in suitable containers, along with the remaining reagents and materials required for the conduct of the assay, as well as a
20 suitable set of assay instructions.

Further Characterization of the HCV Genome, Virions, and
Viral Antigens Using Probes Derived From cDNA to the Viral
25 Genome

The HCV cDNA sequence information in the newly isolated clones described in the Examples may be used to gain further information on the sequence of the HCV genome, and for identification and isolation of the HCV
30 agent, and thus will aid in its characterization including the nature of the genome, the structure of the viral particle, and the nature of the antigens of which it is composed. This information, in turn, can lead to additional polynucleotide probes, polypeptides derived from
35 the HCV genome, and antibodies directed against HCV.

epitopes which would be useful for the diagnosis and/or treatment of HCV caused NANBH.

5 The cDNA sequence information in the above-mentioned clones is useful for the design of probes for the isolation of additional cDNA sequences which are derived from as yet undefined regions of the HCV genome(s) from which the cDNAs in clones described herein and in EP 0,316,218 are derived. For example, labeled probes
10 containing a sequence of approximately 8 or more nucleotides, and preferably 20 or more nucleotides, which are derived from regions close to the 5'-termini or 3'-termini of the composite HCV cDNA sequence shown in Fig. 17 may be used to isolate overlapping cDNA sequences from HCV cDNA
15 libraries. Alternatively, characterization of the genomic segments could be from the viral genome(s) isolated from purified HCV particles. Methods for purifying HCV particles and for detecting them during the purification procedure are described herein, *infra*. Procedures for
20 isolating polynucleotide genomes from viral particles are known in the art, and one procedure which may be used is that described in EP 0,218,316. The isolated genomic segments could then be cloned and sequenced. An example of this technique, which utilizes amplification of the
25 sequences to be cloned, is provided *infra*., and yielded clone 16jh.

 Methods for constructing cDNA libraries are known in the art, and are discussed *supra* and *infra*; a
30 method for the construction of HCV cDNA libraries in lambda-gt11 is discussed in EPO Pub. No. 318,216. However, cDNA libraries which are useful for screening with nucleic acid probes may also be constructed in other vectors known in the art, for example, lambda-gt10 (Huynh et al. (1985)).
35

Screening for Anti-Viral Agents for HCV

5 The availability of cell culture and animal
model systems for HCV makes it possible to screen for
anti-viral agents which inhibit HCV replication, and
particularly for those agents which preferentially allow
cell growth and multiplication while inhibiting viral
replication. These screening methods are known by those
of skill in the art. Generally, the anti-viral agents are
10 tested at a variety of concentrations, for their effect on
preventing viral replication in cell culture systems which
support viral replication, and then for an inhibition of
infectivity or of viral pathogenicity (and a low level of
toxicity) in an animal model system.

15 The methods and compositions provided herein for
detecting HCV antigens and HCV polynucleotides are useful
for screening of anti-viral agents in that they provide an
alternative, and perhaps more sensitive means, for detect-
ing the agent's effect on viral replication than the cell
20 plaque assay or ID₅₀ assay. For example, the HCV-
polynucleotide probes described herein may be used to
quantitate the amount of viral nucleic acid produced in a
cell culture. This could be accomplished, for example, by
hybridization or competition hybridization of the infected
25 cell nucleic acids with a labeled HCV-polynucleotide
probe. For example, also, anti-HCV antibodies may be used
to identify and quantitate HCV antigen(s) in the cell
culture utilizing the immunoassays described herein. In
addition, since it may be desirable to quantitate HCV
30 antigens in the infected cell culture by a competition
assay, the polypeptides encoded within the HCV cDNAs
described herein are useful in these competition assays.
Generally, a recombinant HCV polypeptide derived from the
HCV cDNA would be labeled, and the inhibition of binding
35 of this labeled polypeptide to an HCV polypeptide due to
the antigen produced in the cell culture system would be

monitored. Moreover, these techniques are particularly useful in cases where the HCV may be able to replicate in a cell line without causing cell death.

5 The anti-viral agents which may be tested for efficacy by these methods are known in the art, and include, for example, those which interact with virion components and/or cellular components which are necessary for the binding and/or replication of the virus. Typical
10 anti-viral agents may include, for example, inhibitors of virion polymerase and/or protease(s) necessary for cleavage of the precursor polypeptides. Other anti-viral agents may include those which act with nucleic acids to prevent viral replication, for example, anti-sense
15 polynucleotides, etc.

 Antisense polynucleotides molecules are comprised of a complementary nucleotide sequence which allows them to hybridize specifically to designated regions of genomes or RNAs. Antisense polynucleotides may
20 include, for example, molecules that will block protein translation by binding to mRNA, or may be molecules which prevent replication of viral RNA by transcriptase. They may also include molecules which carry agents (non-covalently attached or covalently bound) which cause the
25 viral RNA to be inactive by causing, for example, scissions in the viral RNA. They may also bind to cellular polynucleotides which enhance and/or are required for viral infectivity, replicative ability, or chronicity. Antisense molecules which are to hybridize to HCV derived
30 RNAs may be designed based upon the sequence information of the HCV cDNAs provided herein. The antiviral agents based upon anti-sense polynucleotides for HCV may be designed to bind with high specificity, to be of increased solubility, to be stable, and to have low toxicity.
35 Hence, they may be delivered in specialized systems, for example, liposomes, or by gene therapy. In addition, they

may include analogs, attached proteins, substituted or altered bonding between bases, etc.

5 Other types of drugs may be based upon polynucleotides which "mimic" important control regions of the HCV genome, and which may be therapeutic due to their interactions with key components of the system responsible for viral infectivity or replication.

10 General Methods

The general techniques used in extracting the genome from a virus, preparing and probing a cDNA library, sequencing clones, constructing expression vectors, trans-
15 forming cells, performing immunological assays such as radioimmunoassays and ELISA assays, for growing cells in culture, and the like are known in the art and laboratory manuals are available describing these techniques. However, as a general guide, the following sets forth some sources currently available for such procedures, and for
20 materials useful in carrying them out.

Both prokaryotic and eukaryotic host cells may be used for expression of desired coding sequences when appropriate control sequences which are compatible with the designated host are used. Among prokaryotic hosts, E. coli
25 coli is most frequently used. Expression control sequences for prokaryotes include promoters, optionally containing operator portions, and ribosome binding sites. Transfer vectors compatible with prokaryotic hosts are commonly derived from, for example, pBR322, a plasmid
30 containing operons conferring ampicillin and tetracycline resistance, and the various pUC vectors, which also contain sequences conferring antibiotic resistance markers. These markers may be used to obtain successful transformants by selection. Commonly used prokaryotic
35 control sequences include the Beta-lactamase (penicillinase) and lactose promoter systems (Chang et al.

(1977)), the tryptophan (*trp*) promoter system (Goeddel et al. (1980)) and the lambda-derived P_L promoter and N gene ribosome binding site (Shimatake et al. (1981)) and the hybrid *tac* promoter (De Boer et al. (1983)) derived from sequences of the *trp* and *lac* UV5 promoters. The foregoing systems are particularly compatible with *E. coli*; if desired, other prokaryotic hosts such as strains of *Bacillus* or *Pseudomonas* may be used, with corresponding control sequences.

Eukaryotic hosts include yeast and mammalian cells in culture systems. *Saccharomyces cerevisiae* and *Saccharomyces carlsbergensis* are the most commonly used yeast hosts, and are convenient fungal hosts. Yeast compatible vectors carry markers which permit selection of successful transformants by conferring prototrophy to auxotrophic mutants or resistance to heavy metals on wild-type strains. Yeast compatible vectors may employ the 2 micron origin of replication (Broach et al. (1983)), the combination of CEN3 and ARS1 or other means for assuring replication, such as sequences which will result in incorporation of an appropriate fragment into the host cell genome. Control sequences for yeast vectors are known in the art and include promoters for the synthesis of glycolytic enzymes (Hess et al. (1968); Holland et al. (1978)), including the promoter for 3 phosphoglycerate kinase (Hitzeman (1980)). Terminators may also be included, such as those derived from the enolase gene (Holland (1981)). Particularly useful control systems are those which comprise the glyceraldehyde-3 phosphate dehydrogenase (GAPDH) promoter or alcohol dehydrogenase (ADH) regulatable promoter, terminators also derived from GAPDH, and if secretion is desired, leader sequence from yeast alpha factor. In addition, the transcriptional regulatory region and the transcriptional initiation region which are operably linked may be such that they are

not naturally associated in the wild-type organism. These systems are described in detail in EPO 120,551, published October 3, 1984; EPO 116,201, published August 22, 1984; and EPO 164,556, published December 18, 1985, all of which are assigned to the herein assignee, and are hereby incorporated herein by reference.

Mammalian cell lines available as hosts for expression are known in the art and include many immortalized cell lines available from the American Type Culture Collection (ATCC), including HeLa cells, Chinese hamster ovary (CHO) cells, baby hamster kidney (BHK) cells, and a number of other cell lines. Suitable promoters for mammalian cells are also known in the art and include viral promoters such as that from Simian Virus 40 (SV40) (Fiers (1978)), Rous sarcoma virus (RSV), adenovirus (ADV), and bovine papilloma virus (BPV). Mammalian cells may also require terminator sequences and poly A addition sequences; enhancer sequences which increase expression may also be included, and sequences which cause amplification of the gene may also be desirable. These sequences are known in the art. Vectors suitable for replication in mammalian cells may include viral replicons, or sequences which insure integration of the appropriate sequences encoding NANBV epitopes into the host genome.

Transformation may be by any known method for introducing polynucleotides into a host cell, including, for example packaging the polynucleotide in a virus and transducing a host cell with the virus, and by direct uptake of the polynucleotide. The transformation procedure used depends upon the host to be transformed. For example, transformation of the E. coli host cells with lambda-gt11 containing BB-NANBV sequences is discussed in the Example section, infra. Bacterial transformation by direct uptake generally employs treatment with calcium or rubidium chloride (Cohen (1972); Maniatis (1982)). Yeast

transformation by direct uptake may be carried out using the method of Hinnen et al. (1978). Mammalian transformations by direct uptake may be conducted using the calcium phosphate precipitation method of Graham and Van der Eb (1978), or the various known modifications thereof.

Vector construction employs techniques which are known in the art. Site-specific DNA cleavage is performed by treating with suitable restriction enzymes under conditions which generally are specified by the manufacturer of these commercially available enzymes. In general, about 1 microgram of plasmid or DNA sequence is cleaved by 1 unit of enzyme in about 20 microliters buffer solution by incubation of 1-2 hr at 37° C. After incubation with the restriction enzyme, protein is removed by phenol/chloroform extraction and the DNA recovered by precipitation with ethanol. The cleaved fragments may be separated using polyacrylamide or agarose gel electrophoresis techniques, according to the general procedures found in Methods in Enzymology (1980) 65:499-560.

Sticky ended cleavage fragments may be blunt ended using E. coli DNA polymerase I (Klenow) in the presence of the appropriate deoxynucleotide triphosphates (dNTPs) present in the mixture. Treatment with S1 nuclease may also be used, resulting in the hydrolysis of any single stranded DNA portions.

Ligations are carried out using standard buffer and temperature conditions using T4 DNA ligase and ATP; sticky end ligations require less ATP and less ligase than blunt end ligations. When vector fragments are used as part of a ligation mixture, the vector fragment is often treated with bacterial alkaline phosphatase (BAP) or calf intestinal alkaline phosphatase to remove the 5'-phosphate and thus prevent religation of the vector; alternatively, restriction enzyme digestion of unwanted fragments can be used to prevent ligation.

Ligation mixtures are transformed into suitable cloning hosts, such as E. coli, and successful transformants selected by, for example, antibiotic resistance, and screened for the correct construction.

Synthetic oligonucleotides may be prepared using an automated oligonucleotide synthesizer as described by Warner (1984). If desired the synthetic strands may be labeled with ^{32}P by treatment with polynucleotide kinase in the presence of ^{32}P -ATP, using standard conditions for the reaction.

DNA sequences, including those isolated from cDNA libraries, may be modified by known techniques, including, for example site directed mutagenesis, as described by Zoller (1982). Briefly, the DNA to be modified is packaged into phage as a single stranded sequence, and converted to a double stranded DNA with DNA polymerase using, as a primer, a synthetic oligonucleotide complementary to the portion of the DNA to be modified, and having the desired modification included in its own sequence. The resulting double stranded DNA is transformed into a phage supporting host bacterium. Cultures of the transformed bacteria, which contain replications of each strand of the phage, are plated in agar to obtain plaques. Theoretically, 50% of the new plaques contain phage having the mutated sequence, and the remaining 50% have the original sequence. Replicates of the plaques are hybridized to labeled synthetic probe at temperatures and conditions which permit hybridization with the correct strand, but not with the unmodified sequence. The sequences which have been identified by hybridization are recovered and cloned.

DNA libraries may be probed using the procedure of Grunstein and Hogness (1975). Briefly, in this procedure, the DNA to be probed is immobilized on nitro-cellulose filters, denatured, and prehybridized with a

buffer containing 0-50% formamide, 0.75 M NaCl, 75 mM Na citrate, 0.02% (wt/v) each of bovine serum albumin, polyvinyl pyrrolidone, and Ficoll, 50 mM Na Phosphate (pH 6.5), 0.1% SDS, and 100 micrograms/ml carrier denatured DNA. The percentage of formamide in the buffer, as well as the time and temperature conditions of the prehybridization and subsequent hybridization steps depends on the stringency required. Oligomeric probes which require lower stringency conditions are generally used with low percentages of formamide, lower temperatures, and longer hybridization times. Probes containing more than 30 or 40 nucleotides such as those derived from cDNA or genomic sequences generally employ higher temperatures, e.g., about 40-42°C, and a high percentage, e.g., 50%, formamide. Following prehybridization, 5'-³²P-labeled oligonucleotide probe is added to the buffer, and the filters are incubated in this mixture under hybridization conditions. After washing, the treated filters are subjected to autoradiography to show the location of the hybridized probe; DNA in corresponding locations on the original agar plates is used as the source of the desired DNA.

For routine vector constructions, ligation mixtures are transformed into E. coli strain HB101 or other suitable host, and successful transformants selected by antibiotic resistance or other markers. Plasmids from the transformants are then prepared according to the method of Clewell et al. (1969), usually following chloramphenicol amplification (Clewell (1972)). The DNA is isolated and analyzed, usually by restriction enzyme analysis and/or sequencing. Sequencing may be by the dideoxy method of Sanger et al. (1977) as further described by Messing et al. (1981), or by the method of Maxam et al. (1980). Problems with band compression, which are sometimes observed in GC rich regions, were

overcome by use of T-deazoguanosine according to Barr et al. (1986).

5 The enzyme-linked immunosorbent assay (ELISA)
can be used to measure either antigen or antibody
concentrations. This method depends upon conjugation of
an enzyme to either an antigen or an antibody, and uses
the bound enzyme activity as a quantitative label. To
10 measure antibody, the known antigen is fixed to a solid
phase (e.g., a microplate or plastic cup), incubated with
test serum dilutions, washed, incubated with anti-
immunoglobulin labeled with an enzyme, and washed again.
Enzymes suitable for labeling are known in the art, and
15 include, for example, horseradish peroxidase. Enzyme
activity bound to the solid phase is measured by adding
the specific substrate, and determining product formation
or substrate utilization colorimetrically. The enzyme
activity bound is a direct function of the amount of anti-
body bound.

20 To measure antigen, a known specific antibody is
fixed to the solid phase, the test material containing
antigen is added, after an incubation the solid phase is
washed, and a second enzyme-labeled antibody is added.
After washing, substrate is added, and enzyme activity is
25 estimated colorimetrically, and related to antigen
concentration.

Examples

30 Described below are examples of the present
invention which are provided only for illustrative
purposes, and not to limit the scope of the present inven-
tion. In light of the present disclosure, numerous
embodiments within the scope of the claims will be appar-
35 ent to those of ordinary skill in the art.

Isolation and Sequence of Overlapping

HCV cDNA Clones 13i, 26j, CA59a, CA84a, CA156e and CA167b

5 The clones 13i, 26j, CA59a, CA84a, CA156e and CA167b were isolated from the lambda-gt11 library which contains HCV cDNA (ATCC No. 40394), the preparation of which is described in EPO Pub. No. 318,216 (published 31 May 1989), and WO 89/04669 (published 1 June 1989).

10 Screening of the library was with the probes described infra., using the method described in Huynh (1985). The frequencies with which positive clones appeared with the respective probes was about 1 in 50,000.

The isolation of clone 13i was accomplished using a synthetic probe derived from the sequence of clone 15 12f. The sequence of the probe was:

5' GAA CGT TGC GAT CTG GAA GAC AGG GAC AGG 3'.

20 The isolation of clone 26j was accomplished using a probe derived from the 5'-region of clone K9-1. The sequence of the probe was:

5' TAT CAG TTA TGC CAA CGG AAG CGG CCC CGA 3'.

25 The isolation procedures for clone 12f and for clone k9-1 (also called K9-1) are described in EPO Pub. No. 318,216, and their sequences are shown in Figs. 1 and 2, respectively. The HCV cDNA sequences of clones 13i and 26j, are shown in Figs. 4 and 5, respectively. Also shown 30 are the amino acids encoded therein, as well as the overlap of clone 13i with clone 12f, and the overlap of clone 26j with clone 13i. The sequences for these clones confirmed the sequence of clone K9-1. Clone K9-1 had been isolated from a different HCV cDNA library (See EP 35 0,218,316).

Clone CA59a was isolated utilizing a probe based upon the sequence of the 5'-region of clone 26j. The sequence of this probe was:

5

5' CTG GTT AGC AGG GCT TTT CTA TCA CCA CAA 3'.

A probe derived from the sequence of clone CA59a was used to isolate clone CA84a. The sequence of the probe used for this isolation was:

10

5' AAG GTC CTG GTA GTG CTG CTA TTT GCC 3'.

Clone CA156e was isolated using a probe derived from the sequence of clone CA84a. The sequence of the probe was:

15

5' ACT GGA CGA CGC AAG GTT GCA ATT GCT CTA 3'.

20

Clone CA167b was isolated using a probe derived from the sequence of clone CA 156e. The sequence of the probe was:

25

5' TTC GAC GTC ACA TCG ATC TGC TTG TCG GGA 3'.

The nucleotide sequences of the HCV cDNAs in clones CA59a, CA84a, CA156e, and CA167b, are shown Figs. 6, 7, 8, and 9, respectively. The amino acids encoded therein, as well as the overlap with the sequences of relevant clones, are also shown in the Figs.

30

Creation of "pi" HCV cDNA Library

A library of HCV cDNA, the "pi" library, was constructed from the same batch of infectious chimpanzee plasma used to construct the lambda-gt11 HCV cDNA library (ATCC No. 40394) described in EPO Pub. No. 318,216, and

35

utilizing essentially the same techniques. However, construction of the pi library utilized a primer-extension method, in which the primer for reverse transcriptase was based on the sequence of clone CA59A. The sequence of the primer was:

5' GGT GAC GTG GGT TTC 3'.

10

Isolation and Sequence of Clone pi14a

Screening of the "pi" HCV cDNA library described supra., with the probe used to isolate clone CA167b (See supra.) yielded clone pi14a. The clone contains about 800 base pairs of cDNA which overlaps clones CA167b, CA156e, CA84a and CA59a, which were isolated from the lambda gt-11 HCV cDNA library (ATCC No. 40394). In addition, pi14a also contains about 250 base pairs of DNA which are upstream of the HCV cDNA in clone CA167b.

20

Isolation and Sequence of Clones CA216a, CA290a and ag30a

Based on the sequence of clone CA167b a synthetic probe was made having the following sequence:

25

5' GGC TTT ACC ACG TCA CCA ATG ATT GCC CTA 3'

The above probe was used to screen the , which yielded clone CA216a, whose HCV sequences are shown in Fig. 10.

Another probe was made based on the sequence of clone CA216a having the following sequence:

30

5' TTT GGG TAA GGT CAT CGA TAC CCT TAC GTG 3'

Screening the lambda-gt11 library (ATCC No. 40394) with this probe yielded clone CA290a, the HCV sequences therein being shown in Fig. 11.

35

In a parallel approach, a primer-extension cDNA library was made using nucleic acid extracted from the same infectious plasma used in the original lambda-gt11 cDNA library described above. The primer used was based on the sequence of clones CA216a and CA290a:

5' GAA GCC GCA CGT AAG 3'

The cDNA library was made using methods similar to those described previously for libraries used in the isolation of clones pil4a and k9-1. The probe used to screen this library was based on the sequence of clone CA290a:

5' CCG GCG TAG GTC GCG CAA TTT GGG TAA 3'

Clone ag30a was isolated from the new library with the above probe, and contained about 670 basepairs of HCV sequence. See Fig. 12. Part of this sequence overlaps the HCV sequence of clones CA216a and CA290a. About 300 base-pairs of the ag30a sequence, however, is upstream of the sequence from clone CA290a. The non-overlapping sequence shows a start codon (*) and stop codons that may indicate the start of the HCV ORF. Also indicated in Fig. 12 are putative small encoded peptides (#) which may play a role in regulating translation, as well as the putative first amino acid of the putative polypeptide (/), and downstream amino acids encoded therein.

Isolation and Sequence of Clone CA205a

Clone CA205a was isolated from the original lambda gt-11 library (ATCC No. 40394), using a synthetic probe derived from the HCV sequence in clone CA290a (Fig. 11). The sequence of the probe was:

5' TCA GAT CGT TGG TGG AGT TTA CTT GTT GCC 3'.

5 The sequence of the HCV cDNA in CA205a, shown in Fig. 13, overlaps with the cDNA sequences in both clones ag30a and CA290a. The overlap of the sequence with that of CA290a is shown by the dotted line above the sequence (the figure also shows the putative amino acids encoded in this fragment).

10 As observed from the HCV cDNA sequences in clones CA205a and ag30a, the putative HCV polyprotein appears to begin at the ATG start codon; the HCV sequences in both clones contain an in-frame, contiguous double stop codon (TGATAG) forty two nucleotides upstream from this ATG. The HCV ORF appears to begin after these stop
15 codons, and to extend for at least 8907 nucleotides (See the composite HCV cDNA shown in Fig. 17).

Isolation and Sequence of Clone 18g

20 Based on the sequence of clone ag30a (See Fig. 12) and of an overlapping clone from the original lambda gt-11 library (ATCC No. 40394), CA230a, a synthetic probe was made having the following sequence:

5' CCA TAG TGG TCT GCG GAA CCG GTG AGT ACA 3'.

25 Screening of the original lambda-gt11 HCV cDNA library with the probe yielded clone 18g, the HCV cDNA sequence of which is shown in Fig. 14. Also shown in the figure are the overlap with clone ag30a, and putative polypeptides encoded within the HCV cDNA.

30 The cDNA in clone 18g (C18g or 18g) overlaps that in clones ag30a and CA205a, described supra. The sequence of C18g also contains the double stop codon region observed in clone ag30a. The polynucleotide region upstream of these stop codons presumably represents part
35 of the 5'-region of the HCV genome, which may contain short ORFs, and which can be confirmed by direct sequenc-

ing of the purified HCV genome. These putative small encoded peptides may play a regulatory role in translation. The region of the HCV genome upstream of that
5 represented by C18g can be isolated for sequence analysis using essentially the technique described in EPO Pub. No. 318,216 for isolating cDNA sequences upstream of the HCV cDNA sequence in clone 12f. Essentially, small synthetic
10 oligonucleotide primers of reverse transcriptase, which are based upon the sequence of C18g, are synthesized and used to bind to the corresponding sequence in HCV genomic RNA. The primer sequences are proximal to the known 5'-terminal of C18g, but sufficiently downstream to allow the
15 design of probe sequences upstream of the primer sequences. Known standard methods of priming and cloning are used. The resulting cDNA libraries are screened with sequences upstream of the priming sites (as deduced from the elucidated sequence of C18g). The HCV genomic RNA is
20 obtained from either plasma or liver samples from individuals with NANBH. Since HCV appears to be a Flavivirus-like virus, the 5'-terminus of the genome may be modified with a "cap" structure. It is known that Flavivirus genomes contain 5'-terminal "cap" structures. (Yellow
25 Fever virus, Rice et al. (1988); Dengue virus, Hahn et al (1988); Japanese Encephalitis Virus (1987)).

Isolation and Sequence of Clones from
the beta-HCV cDNA library

30 Clones containing cDNA representative of the 3'-terminal region of the HCV genome were isolated from a cDNA library constructed from the original infectious chimpanzee plasma pool which was used for the creation of the HCV cDNA lambda-gt11 library (ATCC No. 40394),
35 described in EPO Pub. No. 318,216. In order to create the DNA library, RNA extracted from the plasma was "tailed" with poly rA using poly (rA) polymerase, and cDNA was

synthesized using oligo(dT)₁₂₋₁₈ as a primer for reverse transcriptase. The resulting RNA:CDNA hybrid was digested with RNAase H, and converted to double stranded HCV cDNA. The resulting HCV cDNA was cloned into lambda-gt10, using essentially the technique described in Huynh (1985), yielding the beta (or b) HCV cDNA library. The procedures used were as follows.

10 An aliquot (12ml) of the plasma was treated with proteinase K, and extracted with an equal volume of phenol saturated with 0.05M Tris-Cl, pH 7.5, 0.05% (v/v) beta-mercaptoethanol, 0.1% (w/v) hydroxyquinolone, 1 mM EDTA. The resulting aqueous phase was re-extracted with the phenol mixture, followed by 3 extractions with a 1:1
15 mixture containing phenol and chloroform:isoamyl alcohol (24:1), followed by 2 extractions with a mixture of chloroform and isoamyl alcohol (1:1). Subsequent to adjustment of the aqueous phase to 200 mM with respect to NaCl, nucleic acids in the aqueous phase were precipitated
20 overnight at -20°C, with 2.5 volumes of cold absolute ethanol. The precipitates were collected by centrifugation at 10,000 RPM for 40 min., washed with 70% ethanol containing 20 mM NaCl, and with 100% cold ethanol, dried for 5 min. in a dessicator, and dissolved in water.

25 The isolated nucleic acids from the infectious chimpanzee plasma pool were tailed with poly rA utilizing poly-A polymerase in the presence of human placenta ribonuclease inhibitor (HPRI) (purchased from Amersham Corp.), utilizing MS2 RNA as carrier. Isolated nucleic
30 acids equivalent to that in 2 ml of plasma were incubated in a solution containing TMN (50 mM Tris HCl, pH 7.9, 10 mM MgCl₂, 250 mM NaCl, 2.5 mM MnCl₂, 2 mM dithiothreitol (DTT)), 40 micromolar alpha-[³²P] ATP, 20 units HPRI (Amersham Corp.), and about 9 to 10 units of RNase free
35 poly-A polymerase (BRL). Incubation was for 10 min. at 37°C, and the reactions were stopped with EDTA (final

concentration about 250 mM). The solution was extracted with an equal volume of phenol-chloroform, and with an equal volume of chloroform, and nucleic acids were
5 precipitated overnight at -20°C with 2.5 volumes of ethanol in the presence of 200 mM NaCl.

Isolation of Clone b5a

10 The beta HCV cDNA library was screened by hybridization using a synthetic probe, which had a sequence based upon the HCV cDNA sequence in clone 15e. The isolation of clone 15e is described in EPO Pub. No. 318,216, and its sequence is shown in Fig. 3. The
15 sequence of the synthetic probe was:

5' ATT GCG AGA TCT ACG GGG CCT GCT ACT CCA 3'.

Screening of the library yielded clone beta-5a (b5a), which contains an HCV cDNA region of approximately 1000
20 base pairs. The 5'-region of this cDNA overlaps clones 35f, 19g, 26g, and 15e (these clones are described supra). The region between the 3'-terminal poly-A sequence and the 3'-sequence which overlaps clone 15e, contains ap-
25 proximately 200 base pairs. This clone allows the identification of a region of the 3'-terminal sequence the HCV genome.

The sequence of b5a is contained within the sequence of the HCV cDNA in clone 16jh (described infra). Moreover, the sequence is also present in CC34a, isolated
30 from the original lambda-gt11 library (ATCC No. 40394). (The original lambda-gt11 library is referred to herein as the "C" library).

35

Isolation and Sequence of Clones Generated by PCR
Amplification of the 3'-Region of the HCV Genome

Multiple cDNA clones have been generated which contain nucleotide sequences derived from the 3'-region of the HCV genome. This was accomplished by amplifying a targeted region of the genome by a polymerase chain reaction technique described in Saiki et al. (1986), and in Saiki et al. (1988), which was modified as described below. The HCV RNA which was amplified was obtained from the original infectious chimpanzee plasma pool which was used for the creation of the HCV cDNA lambda-gt11 library (ATCC No. 40394) described in EPO Pub. No. 318,216. Isolation of the HCV RNA was as described supra. The isolated RNA was tailed at the 3'-end with ATP by E. coli poly-A polymerase as described in Sippel (1973), except that the nucleic acids isolated from chimp serum were substituted for the nucleic acid substrate. The tailed RNA was then reverse transcribed into cDNA by reverse transcriptase, using an oligo dT-primer adapter, essentially as described by Han (1987), except that the components and sequence of the primer-adapter were:

| | | | | |
|----|----------------|-------------|----------------------------|-----------------|
| 25 | <u>Stuffer</u> | <u>NotI</u> | <u>SP6 Promoter</u> | <u>Primer</u> |
| | AATTC | GCGGCCGC | CATACGATTTAGGTGACACTATAGAA | T ₁₅ |

The resultant cDNA was subjected to amplification by PCR using two primers:

| | | |
|----|---------------|--------------------------------|
| 30 | <u>Primer</u> | <u>Sequence</u> |
| | JH32 (30mer) | ATAGCGGCCGCCCTCGATTGCGAGATCTAC |
| | JH11 (20mer) | AATTCGGGCGGCCGCCATACGA |

The JH32 primer contained 20 nucleotide sequences hybridizable to the 5'-end of the target region in the cDNA, with an estimated T_m of 66°C. The JH11 was derived

from a portion of the oligo dT-primer adapter; thus, it is specific to the 3'-end of the cDNA with a T_m of 64°C.

Both primers were designed to have a recognition site for the restriction enzyme, NotI, at the 5'-end, for use in subsequent cloning of the amplified HCV cDNA.

The PCR reaction was carried out by suspending the cDNA and the primers in 100 microliters of reaction mixture containing the four deoxynucleoside triphosphates, buffer salts and metal ions, and a thermostable DNA polymerase isolated from Thermus aquaticus (Taq polymerase), which are in a Perkin Elmer Cetus PCR kit (N801-0043 or N801-0055). The PCR reaction was performed for 35 cycles in a Perkin Elmer Cetus DNA thermal cycler. Each cycle consisted of a 1.5 min denaturation step at 94°C, an annealing step at 60°C for 2 min, and a primer extension step at 72°C for 3 min. The PCR products were subjected to Southern blot analysis using a 30 nucleotide probe, JH34, the sequence of which was based upon that of the 3'-terminal region of clone 15e. The sequence of JH34 is:

5' CTT GAT CTA CCT CCA ATC ATT CAA AGA CTC 3'.

The PCR products detected by the HCV cDNA probe ranged in size from about 50 to about 400 base pairs.

In order to clone the amplified HCV cDNA, the PCR products were cleaved with NotI and size selected by polyacrylamide gel electrophoresis. DNA larger than 300 base pairs was cloned into the NotI site of pUC18S. The vector pUC18S is constructed by including a NotI polylinker cloned between the EcoRI and SalI sites of pUC18. The clones were screened for HCV cDNA using the JH34 probe. A number of positive clones were obtained and sequenced. The nucleotide sequence of the HCV cDNA insert in one of these clones, 16jh, and the amino acids encoded

therein, are shown in Fig. 15. A nucleotide heterogeneity, detected in the sequence of the HCV cDNA in clone 16jh as compared to another clone of this region, is indicated in the figure.

Compiled HCV cDNA Sequences

An HCV cDNA sequence has been compiled from a series of overlapping clones derived from the various HCV cDNA libraries described supra.. In this sequence, the compiled HCV cDNA sequence obtained from clones b114a, 18g, ag30a, CA205a, CA290a, CA216a, p114a, CA167b, CA156e, CA84a, and CA59a is upstream of the compiled HCV cDNA sequence published in EPO Pub. No. 318,216, which is shown in Fig. 16. The compiled HCV cDNA sequence obtained from clones b5a and 16jh downstream of the compiled HCV cDNA sequence published in EPO Pub. No. 318,216.

Fig. 17 shows the compiled HCV cDNA sequence derived from the above-described clones and the compiled HCV cDNA sequence published in EPO Pub. No. 318,216. The clones from which the sequence was derived are b114a, 18g, ag30a, CA205a, CA290a, CA216a, p114a, CA167b, CA156e, CA84a, CA59a, K9-1 (also called k9-1), 26j, 13i, 12f, 14i, 11b, 7f, 7e, 8h, 33c, 40b, 37b, 35, 36, 81, 32, 33b, 25c, 14c, 8f, 33f, 33g, 39c, 35f, 19g, 26g, 15e, b5a, and 16jh. In the figure the three dashes above the sequence indicate the position of the putative initiator methionine codon.

Clone b114a was obtained using the cloning procedure described for clone b5a, supra., except that the probe was the synthetic probe used to detect clone 18g, supra. Clone b114a overlaps with clones 18g, ag30a, and CA205a, except that clone b114a contains an extra two nucleotides upstream of the sequence in clone 18g (i.e., 5'-CA). These extra two nucleotides have been included in the HCV genomic sequence shown in Fig. 17.

It should be noted that although several of the clones described supra. have been obtained from libraries other than the original HCV cDNA lambda-gt11 C library (ATCC No. 40394), these clones contain HCV cDNA sequences which overlap HCV cDNA sequences in the original library. Thus, essentially all of the HCV sequence is derivable from the original lambda-gt11 C library (ATCC No. 40394) which was used to isolate the first HCV cDNA clone (5-1-1). The isolation of clone 5-1-1 is described in EPO Pub. No. 318,216.

Purification of Fusion Polypeptide C100-3

(Alternate method)

The fusion polypeptide, C100-3 (also called HCV c100-3 and alternatively, c100-3), is comprised of superoxide dismutase (SOD) at the N-terminus an in-frame C100 HCV polypeptide at the C-terminus. A method for preparing the polypeptide by expression in yeast, and differential extraction of the insoluble fraction of the extracted host yeast cells, is described in EPO Pub. No. 318,216. An alternative method for the preparation of this fusion polypeptide is described below. In this method the antigen is precipitated from the crude cell lysate with acetone; the acetone precipitated antigen is then subjected to ion-exchange chromatography, and further purified by gel filtration.

The fusion polypeptide, C100-3 (HCV c100-3), is expressed in yeast strain JSC 308 (ATCC No. 20879) transformed with pAB24C100-3 (ATCC No. 67976); the transformed yeast are grown under conditions which allow expression (i.e., by growth in YEP containing 1% glucose). (See EPO Pub. No. 318,216). A cell lysate is prepared by suspending the cells in Buffer A (20 mM Tris HCl, pH 8.0, 1 mM EDTA, 1 mM PMSF. The cells are broken by grinding with glass beads in a Dynomill type homogenizer or its

equivalent. The extent of cell breakage is monitored by counting cells under a microscope with phase optics.

5 Broken cells appear dark, while viable cells are light-colored. The percentage of broken cells is determined.

When the percentage of broken cells is approximately 90% or greater, the broken cell debris is separated from the glass beads by centrifugation, and the glass beads are washed with Buffer A. After combining the
10 washes and homogenate, the insoluble material in the lysate is obtained by centrifugation. The material in the pellet is washed to remove soluble proteins by suspension in Buffer B (50 mM glycine, pH 12.0, 1 mM DTT, 500 mM NaCl), followed by Buffer C (50 mM glycine, pH 10.0, 1 mM
15 DTT). The insoluble material is recovered by centrifugation, and solubilized by suspension in Buffer C containing SDS. The extract solution may be heated in the presence of beta-mercaptoethanol and concentrated by ultrafiltration. The HCV c100-3 in the extract is
20 precipitated with cold acetone. If desired, the precipitate may be stored at temperatures at about or below -15°C.

Prior to ion exchange chromatography, the acetone precipitated material is recovered by centrifuga-
25 tion, and may be dried under nitrogen. The precipitate is suspended in Buffer D (50 mM glycine, pH 10.0, 1 mM DTT, 7 M urea), and centrifuged to pellet insoluble material. The supernatant material is applied to an anion exchange column previously equilibrated with Buffer D. Fractions
30 are collected and analyzed by ultraviolet absorbance or gel electrophoresis on SDS polyacrylamide gels. Those fractions containing the HCV c100-3 polypeptide are pooled.

In order to purify the HCV c100-3 polypeptide by
35 gel filtration, the pooled fractions from the ion-exchange column are heated in the presence of beta-mercaptoethanol

and SDS, and the eluate is concentrated by ultrafiltration. The concentrate is applied to a gel filtration column previously equilibrated with Buffer E (20 mM Tris HCl, pH 7.0, 1 mM DTT, 0.1% SDS). The presence of HCV c100-3 in the eluted fractions, as well as the presence of impurities, are determined by gel electrophoresis on polyacrylamide gels in the presence of SDS and visualization of the polypeptides. Those fractions containing purified HCV c100-3 are pooled. Fractions high in HCV c100-3 may be further purified by repeating the gel filtration process. If the removal of particulate material is desired, the HCV c100-3 containing material may be filtered through a 0.22 micron filter.

Expression and Antigenicity of Polypeptides
Encoded in HCV cDNA

Polypeptides Expressed in E. coli

The polypeptides encoded in a number of HCV cDNAs which span the HCV genomic ORF were expressed in E. coli, and tested for their antigenicity using serum obtained from a variety of individuals with NANBH. The expression vectors containing the cloned HCV cDNAs were constructed from pSODcfl (Steimer et al. (1986). In order to be certain that a correct reading frame would be achieved, three separate expression vectors, pcflAB, pcflCD, and pcflEF were created by ligating either of three linkers, AB, CD, and EF to a BamHI-EcoRI fragment derived by digesting to completion the vector pSODcfl with EcoRI and BamHI, followed by treatment with alkaline phosphatase. The linkers were created from six oligomers, A, B, C, D, E, and F. Each oligomer was phosphorylated by treatment with kinase in the presence of ATP prior to annealing to its complementary oligomer. The sequences of the synthetic linkers were the following.

| | Name | DNA Sequence (5' to 3') |
|----|------|---------------------------|
| 5 | A | GATC CTG AAT TCC TGA TAA |
| | B | GAC TTA AGG ACT ATT TTA A |
| | C | GATC CGA ATT CTG TGA TAA |
| 10 | D | GCT TAA GAC ACT ATT TTA A |
| | E | GATC CTG GAA TTC TGA TAA |
| | F | GAC CTT AAG ACT ATT TTA A |

15 Each of the three linkers destroys the original EcoRI site, and creates a new EcoRI site within the linker, but within a different reading frame. Hence, the HCV cDNA EcoRI fragments isolated from the clones when inserted into the expression vector, were in three different reading frames.

20 The HCV cDNA fragments in the designated lambda-gt11 clones were excised by digestion with EcoRI; each fragment was inserted into pcflAB, pcflCD, and pcflEF. These expression constructs were then transformed into D1210 E. coli cells, the transformants were cloned, and
25 recombinant bacteria from each clone were induced to express the fusion polypeptides by growing the bacteria in the presence of IPTG.

30 Expression products of the indicated HCV cDNAs were tested for antigenicity by direct immunological screening of the colonies, using a modification of the method described in Helfman et al. (1983). Briefly, as shown in Fig. 18, the bacteria were plated onto nitro-cellulose filters overlaid on ampicillin plates to give approximately 1,000 colonies per filter. Colonies were
35 replica plated onto nitrocellulose filters, and the replicas were regrown overnight in the presence of 2 mM

5 IPTG and ampicillin. The bacterial colonies were lysed by
suspending the nitrocellulose filters for about 15 to 20
min in an atmosphere saturated with CHCl_3 vapor. Each
10 filter then was placed in an individual 100 mm Petri dish
containing 10 ml of 50 mM Tris HCl, pH 7.5, 150 mM NaCl, 5
mM MgCl_2 , 3% (w/v) BSA, 40 micrograms/ml lysozyme, and 0.1
microgram/ml DNase. The plates were agitated gently for
at least 8 hours at room temperature. The filters were
15 rinsed in TBST (50 mM Tris HCl, pH8.0, 150 mM NaCl, 0.005%
Tween 20). After incubation, the cell residues were
rinsed and incubated in TBS (TBST without Tween) contain-
ing 10% sheep serum; incubation was for 1 hour. The
filters were then incubated with pretreated sera in TBS
20 from individuals with NANBH, which included: 3
chimpanzees; 8 patients with chronic NANBH whose sera were
positive with respect to antibodies to HCV C100-3
polypeptide (described in EPO Pub. No. 318,216, and
supra.) (also called C100); 8 patients with chronic NANBH
25 whose sera were negative for anti-C100 antibodies; a
convalescent patient whose serum was negative for anti-
C100 antibodies; and 6 patients with community acquired
NANBH, including one whose sera was strongly positive with
respect to anti-C100 antibodies, and one whose sera was
30 marginally positive with respect to anti-C100 antibodies.
The sera, diluted in TBS, was pretreated by preabsorption
with hSOD. Incubation of the filters with the sera was
for at least two hours. After incubation, the filters
were washed two times for 30 min with TBST. Labeling of
35 expressed proteins to which antibodies in the sera bound
was accomplished by incubation for 2 hours with ^{125}I -
labeled sheep anti-human antibody. After washing, the
filters were washed twice for 30 min with TBST, dried, and
autoradiographed.

A number of clones (see infra.) expressed
polypeptides containing HCV epitopes which were im-

munologically reactive with serum from individuals with NANBH. Five of these polypeptides were very immunogenic in that antibodies to HCV epitopes in these polypeptides were detected in many different patient sera. The clones encoding these polypeptides, and the location of the polypeptide in the putative HCV polyprotein (wherein the amino acid numbers begin with the putative initiator codon) are the following: clone 5-1-1, amino acids 1694-1735; clone C100, amino acids 1569-1931; clone 33c, amino acids 1192-1457; clone CA279a, amino acids 1-84; and clone CA290a amino acids 9-177. The location of the immunogenic polypeptides within the putative HCV polyprotein are shown immediately below.

15

20

25

30

35

5 Clones encoding polypeptides of proven reactivity
 with sera from NANBH patients.

| | <u>Clone</u> | <u>Location within the HCV polyprotein</u> (amino acid no. beginning with puta- tive initiator methionine) |
|----|--------------|--|
| 10 | | |
| | CA279a | 1-84 |
| | CA74a | 437-582 |
| | 13i | 511-690 |
| | CA290a | 9-177 |
| 15 | 33c | 1192-1457 |
| | 40b | 1266-1428 |
| | 5-1-1 | 1694-1735 |
| | 81 | 1689-1805 |
| | 33b | 1916-2021 |
| 20 | 25c | 1949-2124 |
| | 14c | 2054-2223 |
| | 8f | 2200-3325 |
| | 33f | 2287-2385 |
| | 33g | 2348-2464 |
| 25 | 39c | 2371-2502 |
| | 15e | 2796-2886 |
| | C100 | 1569-1931 |

30 The results on the immunogenicity of the
 polypeptides encoded in the various clones examined sug-
 gest efficient detection and immunization systems may
 include panels of HCV polypeptides/epitopes.

35 Expression of HCV Epitopes in Yeast

 Three different yeast expression vectors which
 allow the insertion of HCV cDNA into three different read-

ing frames are constructed. The construction of one of the vectors, pAB24C100-3 is described in EPO Pub. No. 318,216. In the studies below, the HCV cDNA from the clones listed in supra. in the antigenicity mapping study using the E. coli expressed products are substituted for the C100 HCV cDNA. The construction of the other vectors replaces the adaptor described in the above E. coli studies with one of the following adaptors:

Adaptor 1

ATT TTG AAT TCC TAA TGA G
AC TTA AGG ATT ACT CAG CT

Adaptor 2

AAT TTG GAA TTC TAA TGA G
AC CTT AAG ATT ACT CAG CT.

The inserted HCV cDNA is expressed in yeast transformed with the vectors, using the expression conditions described supra. for the expression of the fusion polypeptide, C100-3. The resulting polypeptides are screened using the sera from individuals with NANBH, described supra. for the screening of immunogenic polypeptides encoded in HCV cDNAs expressed in E. coli.

Comparison of the Hydrophobic Profiles of HCV Polyproteins with West Nile Virus Polyprotein and with Dengue Virus NS1

The hydrophobicity profile of an HCV polyprotein segment was compared with that of a typical Flavivirus, West Nile virus. The polypeptide sequence of the West Nile virus polyprotein was deduced from the known polynucleotide sequences encoding the non-structural

proteins of that virus. The HCV polyprotein sequence was deduced from the sequence of overlapping cDNA clones. The profiles were determined using an antigen program which
5 uses a window of 7 amino acid width (the amino acid in question, and 3 residues on each side) to report the average hydrophobicity about a given amino acid residue. The parameters giving the reactive hydrophobicity for each amino acid residue are from Kyte and Doolittle (1982).
10 Fig. 19 shows the hydrophobic profiles of the two polyproteins; the areas corresponding to the non-structural proteins of West Nile virus, ns1 through ns5, are indicated in the figure. As seen in the figure, there is a general similarity in the profiles of the HCV
15 polyprotein and the West Nile virus polyprotein.

The sequence of the amino acids encoded in the 5'-region of HCV cDNA shown in Fig. 16 has been compared with the corresponding region of one of the strains of Dengue virus, described supra., with respect to the
20 profile of regions of hydrophobicity and hydrophilicity (data not shown). This comparison indicated that the polypeptides from HCV and Dengue encoded in this region, which corresponds to the region encoding NS1 (or a portion thereof), have a similar hydrophobic/hydrophilic profile.
25

The similarity in hydrophobicity profiles, in combination with the previously identified homologies in the amino acid sequences of HCV and Dengue Flavivirus in EP 0,218,316 suggests that HCV is related to these members of the Flavivirus family.
30

Characterization of the Putative Polypeptides Encoded Within the HCV ORF

The sequence of the HCV cDNA sense strand, shown in Fig. 17, was deduced from the overlapping HCV cDNAs in
35 the various clones described in EPO Pub. No. 318,216 and those described supra. It may be deduced from the

sequence that the HCV genome contains primarily one long continuous ORF, which encodes a polyprotein. In the sequence, nucleotide number 1 corresponds to the first
5 nucleotide of the initiator MET codon; minus numbers indicate that the nucleotides are that distance away in the 5'-direction (upstream), while positive numbers indicate that the nucleotides are that distance away in the 3'-direction (downstream). The composite sequence
10 shows the "sense" strand of the HCV cDNA.

The amino acid sequence of the putative HCV polyprotein deduced from the HCV cDNA sense strand sequence is also shown in Fig. 17, where position 1 begins with the putative initiator methionine.

15 Possible protein domains of the encoded HCV polyprotein, as well as the approximate boundaries, are the following (the polypeptides identified within the parentheses are those which are encoded in the Flavivirus domain):

| | <u>Putative Domain</u> | <u>Approximate Boundary</u> <u>(amino acid nos.)</u> |
|----|--|---|
| 25 | "C" (nucleocapsid protein) | 1-120 |
| | "E" (Virion envelope protein(s) and possibly matrix (M) proteins | 120-400 |
| 30 | "NS1" (complement fixation antigen?) | 400-660 |
| | "NS2" (unknown function) | 660-1050 |
| 35 | "NS3" (protease?) | 1050-1640 |

"NS4" (unknown function)

1640-2000

"NS5" (polymerase)

2000-? end

5

It should be noted, however, that hydrophobicity profiles (described infra), indicate that HCV diverges from the Flavivirus model, particularly with respect to the region upstream of NS2. Moreover, the boundaries indicated are not intended to show firm demarcations between the putative polypeptides.

15

The Hydrophilic and Antigenic Profile of the Polypeptide

Profiles of the hydrophilicity/hydrophobicity and the antigenic index of the putative polyprotein encoded in the HCV cDNA sequence shown in Fig. 16 were determined by computer analysis. The program for hydrophilicity/hydrophobicity was as described supra. The antigenic index results from a computer program which relies on the following criteria: 1) surface probability, 2) prediction of alpha-helicity by two different methods; 3) prediction of beta-sheet regions by two different methods; 4) prediction of U-turns by two different methods; 5) hydrophilicity/hydrophobicity; and flexibility. The traces of the profiles generated by the computer analyses are shown in Fig. 20. In the hydrophilicity profile, deflection above the abscissa indicates hydrophilicity, and below the abscissa indicates hydrophobicity. The probability that a polypeptide region is antigenic is usually considered to increase when there

is a deflection upward from the abscissa in the hydrophilic and/or antigenic profile. It should be noted, however, that these profiles are not necessarily indicators of the strength of the immunogenicity of a polypeptide.

Identification of Co-linear Peptides in HCV and Flaviviruses

The amino acid sequence of the putative polyprotein encoded in the HCV cDNA sense strand was compared with the known amino acid sequences of several members of Flaviviruses. The comparison shows that homology is slight, but due to the regions in which it is found, it is probably significant. The conserved co-linear regions are shown in Fig. 21. The amino acid numbers listed below the sequences represent the number in the putative HCV polyprotein (See Fig. 17.)

The spacing of these conserved motifs is similar between the Flaviviruses and HCV, and implies that there is some similarity between HCV and these flaviviral agents.

The following listed materials are on deposit under the terms of the Budapest Treaty with the American Type Culture Collection (ATCC), 12301 Parklawn Dr., Rockville, Maryland 20852, and have been assigned the following Accession Numbers.

35

| | <u>lambda-gt11</u> | <u>ATCC No.</u> | <u>Deposit Date</u> |
|----|--------------------|-----------------|---------------------|
| | HCV cDNA library | 40394 | 1 Dec. 1987 |
| 5 | clone 81 | 40388 | 17 Nov. 1987 |
| | clone 91 | 40389 | 17 Nov. 1987 |
| | clone 1-2 | 40390 | 17 Nov. 1987 |
| | clone 5-1-1 | 40391 | 18 Nov. 1987 |
| | clone 12f | 40514 | 10 Nov. 1988 |
| | clone 35f | 40511 | 10 Nov. 1988 |
| 10 | clone 15e | 40513 | 10 Nov. 1988 |
| | clone K9-1 | 40512 | 10 Nov. 1988 |
| | JSC 308 | 20879 | 5 May 1988 |
| | pS356 | 67683 | 29 April 1988 |

15 In addition, the following deposits were made on 11 May 1989.

| | <u>Strain</u> | <u>Linkers</u> | <u>ATCC No.</u> |
|----|-----------------------|----------------|-----------------|
| 20 | D1210 (Cf1/5-1-1) | EF | 67967 |
| | D1210 (Cf1/81) | EF | 67968 |
| | D1210 (Cf1/CA74a) | EF | 67969 |
| | D1210 (Cf1/35f) | AB | 67970 |
| | D1210 (Cf1/279a) | EF | 67971 |
| | D1210 (Cf1/C36) | CD | 67972 |
| 25 | D1210 (Cf1/13i) | AB | 67973 |
| | D1210 (Cf1/C33b) | EF | 67974 |
| | D1210 (Cf1/CA290a) | AB | 67975 |
| | HB101 (AB24/C100 #3R) | | 67976 |

30 The following derivatives of strain D1210 were deposited on 3 May 1989.

35

| | <u>Strain Derivative</u> | <u>ATCC No.</u> |
|----|--------------------------|-----------------|
| | pCF1CS/C8f | 67956 |
| 5 | pCF1AB/C12f | 67952 |
| | pCF1EF/14c | 67949 |
| | pCF1EF/15e | 67954 |
| | pCF1AB/C25c | 67958 |
| | pCF1EF/C33c | 67953 |
| 10 | pCF1EF/C33f | 67050 |
| | pCF1CD/33g | 67951 |
| | pCF1CD/C39c | 67955 |
| | pCF1EF/C40b | 67957 |
| | pCF1EF/CA167b | 67959 |

15 The following strains were deposited on May 12, 1989.

| | <u>Strain</u> | <u>ATCC No.</u> |
|----|----------------------|-----------------|
| | Lambda gt11(C35) | 40603 |
| 20 | Lambda gt10(beta-5a) | 40602 |
| | D1210 (C40b) | 67980 |
| | D1210 (M16) | 67981 |

25 Upon allowance and issuance of this application as a
United States Patent, all restriction on availability of
these deposits will be irrevocably removed; and access to
the designated deposits will be available during pendency
of the above-named application to one determined by the
Commissioner to be entitled thereto under 37 CFR 1.14 and
30 35 USC 1.22. Moreover, the designated deposits will be
maintained for a period of thirty (30) years from the date
of deposit, or for five (5) years after the last request
for the deposit; or for the enforceable life of the U.S.
patent, whichever is longer. The deposited materials
35 mentioned herein are intended for convenience only, and
are not required to practice the present invention in view

of the descriptions herein, and in addition these materials are incorporated herein by reference.

5

Industrial Applicability

The invention, in the various manifestations disclosed herein, has many industrial uses, some of which are the following. The HCV cDNAs may be used for the design of probes for the detection of HCV nucleic acids in
10 samples. The probes derived from the cDNAs may be used to detect HCV nucleic acids in, for example, chemical synthetic reactions. They may also be used in screening programs for anti-viral agents, to determine the effect of the agents in inhibiting viral replication in cell culture
15 systems, and animal model systems. The HCV polynucleotide probes are also useful in detecting viral nucleic acids in humans, and thus, may serve as a basis for diagnosis of HCV infections in humans.

In addition to the above, the cDNAs provided
20 herein provide information and a means for synthesizing polypeptides containing epitopes of HCV. These polypeptides are useful in detecting antibodies to HCV antigens. A series of immunoassays for HCV infection, based on recombinant polypeptides containing HCV epitopes
25 are described herein, and will find commercial use in diagnosing HCV induced NANBH, in screening blood bank donors for HCV-caused infectious hepatitis, and also for detecting contaminated blood from infectious blood donors. The viral antigens will also have utility in monitoring
30 the efficacy of anti-viral agents in animal model systems. In addition, the polypeptides derived from the HCV cDNAs disclosed herein will have utility as vaccines for treatment of HCV infections.

The polypeptides derived from the HCV cDNAs,
35 besides the above stated uses, are also useful for raising anti-HCV antibodies. Thus, they may be used in anti-HCV

5 vaccines. However, the antibodies produced as a result of immunization with the HCV polypeptides are also useful in detecting the presence of viral antigens in samples. Thus, they may be used to assay the production of HCV polypeptides in chemical systems. The anti-HCV antibodies may also be used to monitor the efficacy of anti-viral agents in screening programs where these agents are tested in tissue culture systems. They may also be used for pas-
10 sive immunotherapy, and to diagnose HCV caused NANBH by allowing the detection of viral antigen(s) in both blood donors and recipients. Another important use for anti-HCV antibodies is in affinity chromatography for the purification of virus and viral polypeptides. The purified virus
15 and viral polypeptide preparations may be used in vaccines. However, the purified virus may also be useful for the development of cell culture systems in which HCV replicates.

20 Antisense polynucleotides may be used as inhibitors of viral replication.

For convenience, the anti-HCV antibodies and HCV polypeptides, whether natural or recombinant, may be packaged into kits.

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CLAIMS

5 1. A recombinant polynucleotide comprising a
sequence derived from HCV cDNA, wherein the HCV cDNA is in
clone 13i, or clone 26j, or clone 59a, or clone 84a, or
clone CA156e, or clone 167b, or clone pil4a, or clone
CA216a, or clone CA290a, or clone ag30a, or clone 205a, or
10 clone 18g, or clone 16jh, or wherein the HCV cDNA is of a
sequence indicated by nucleotide numbers -319 to 1348 or
8659 to 8866 in Fig. 17.

15 2. A recombinant polynucleotide according to
claim 1, encoding an epitope of HCV.

 3. A recombinant vector comprising the
polynucleotide of claim 1 or claim 2.

20 4. A host cell transformed with the vector of
claim 3.

25 5. A recombinant expression system comprising
an open reading frame (ORF) of DNA derived from the re-
combinant polynucleotide of claim 1 or claim 2, wherein
the ORF is operably linked to a control sequence compat-
ible with a desired host.

30 6. A cell transformed with the recombinant
expression system of claim 5.

 7. A polypeptide produced by the cell of claim
6.

35 8. A purified polypeptide comprising an epitope
encoded within HCV cDNA wherein the HCV cDNA is of a

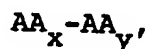
sequence indicated by nucleotide numbers -319 to 1348 or 8659 to 8866 in Fig. 17.

5 9. An immunogenic polypeptide produced by a cell transformed with a recombinant expression vector comprising an ORF of DNA derived from HCV cDNA, wherein the HCV cDNA is comprised of a sequence derived from the HCV cDNA sequence in clone CA279a, or clone CA74a, or
10 clone 13i, or clone CA290a, or clone 33C or clone 40b, or clone 33b, or clone 25c, or clone 14c, or clone 8f, or clone 33f, or clone 33g, or clone 39c, or clone 15e, and wherein the ORF is operably linked to a control sequence compatible with a desired host.

15

10. A peptide comprising an HCV epitope, wherein the peptide is of the formula

20



wherein x and y designate amino acid numbers shown in Fig. 17, and wherein the peptide is selected from the group consisting of AA1-AA25, AA1-AA50, AA1-AA84, AA9-AA177, AA1-AA10, AA5-AA20, AA20-AA25, AA35-AA45, AA50-AA100,
25 AA40-AA90, AA45-AA65, AA65-AA75, AA80-90, AA99-AA120, AA95-AA110, AA105-AA120, AA100-AA150, AA150-AA200, AA155-AA170, AA190-AA210, AA200-AA250, AA220-AA240, AA245-AA265, AA250-AA300, AA290-AA330, AA290-305, AA300-AA350, AA310-AA330, AA350-AA400, AA380-AA395,
30 AA405-AA495, AA400-AA450, AA405-AA415, AA415-AA425, AA425-AA435, AA437-AA582, AA450-AA500, AA440-AA460, AA460-AA470, AA475-AA495, AA500-AA550, AA511-AA690, AA515-AA550, AA550-AA600, AA550-AA625, AA575-AA605, AA585-AA600, AA600-AA650, AA600-AA625, AA635-AA665, AA650-AA700,
35 AA645-AA680, AA700-AA750, AA700-AA725, AA700-AA750, AA725-AA775, AA770-AA790, AA750-AA800, AA800-AA815,

AA825-AA850, AA850-AA875, AA800-AA850, AA920-AA990,
AA850-AA900, AA920-AA945, AA940-AA965, AA970-AA990,
AA950-AA1000, AA1000-AA1060, AA1000-AA1025, AA1000-AA1050,
5 AA1025-AA1040, AA1040-AA1055, AA1075-AA1175,
AA1050-AA1200, AA1070-AA1100, AA1100-AA1130,
AA1140-AA1165, AA1192-AA1457, AA1195-AA1250,
AA1200-AA1225, AA1225-AA1250, AA1250-AA1300,
AA1260-AA1310, AA1260-AA1280, AA1266-AA1428,
10 AA1300-AA1350, AA1290-AA1310, AA1310-AA1340, AA1345-
AA1405, AA1345-AA1365, AA1350-AA1400, AA1365-AA1380,
AA1380-AA1405, AA1400-AA1450, AA1450-AA1500,
AA1460-AA1475, AA1475-AA1515, AA1475-AA1500,
AA1500-AA1550, AA1500-AA1515, AA1515-AA1550,
15 AA1550-AA1600, AA1545-AA1560, AA1569-AA1931,
AA1570-AA1590, AA1595-AA1610, AA1590-AA1650,
AA1610-AA1645, AA1650-AA1690, AA1685-AA1770,
AA1689-AA1805, AA1690-AA1720, AA1694-AA1735,
AA1720-AA1745, AA1745-AA1770, AA1750-AA1800,
20 AA1775-AA1810, AA1795-AA1850, AA1850-AA1900,
AA1900-AA1950, AA1900-AA1920, AA1916-AA2021,
AA1920-AA1940, AA1949-AA2124, AA1950-AA2000,
AA1950-AA1985, AA1980-AA2000, AA2000-AA2050,
AA2005-AA2025, AA2020-AA2045, AA2045-AA2100,
25 AA2045-AA2070, AA2054-AA2223, AA2070-AA2100,
AA2100-AA2150, AA2150-AA2200, AA2200-AA2250,
AA2200-AA2325, AA2250-AA2330, AA2255-AA2270,
AA2265-AA2280, AA2280-AA2290, AA2287-AA2385,
AA2300-AA2350, AA2290-AA2310, AA2310-AA2330,
30 AA2330-AA2350, AA2350-AA2400, AA2348-AA2464,
AA2345-AA2415, AA2345-AA2375, AA2370-AA2410,
AA2371-AA2502, AA2400-AA2450, AA2400-AA2425,
AA2415-AA2450, AA2445-AA2500, AA2445-AA2475,
AA2470-AA2490, AA2500-AA2550, AA2505-AA2540,
35 AA2535-AA2560, AA2550-AA2600, AA2560-AA2580,
AA2600-AA2650, AA2605-AA2620, AA2620-AA2650,

AA2640-AA2660, AA2650-AA2700, AA2655-AA2670,
AA2670-AA2700, AA2700-AA2750, AA2740-AA2760,
AA2750-AA2800, AA2755-AA2780,
5 AA2780-AA2830, AA2785-AA2810, AA2796-AA2886,
AA2810-AA2825, AA2800-AA2850, AA2850-AA2900,
AA2850-AA2865, AA2885-AA2905, AA2900-AA2950,
AA2910-AA2930, AA2925-AA2950, AA2945-end(C' terminal).

10 11. A polypeptide comprised of the peptide of
claim 10.

12. An immunogenic polypeptide attached to a
solid substrate, wherein the polypeptide is according to
15 claim 7, or claim 8, or claim 9, or claim 10, or claim 11,
or wherein the polypeptide is comprised of an epitope
encoded within HCV cDNA wherein the HCV cDNA is of a
sequence indicated by nucleotide numbers -319 to 1348 or
8659 to 8866 in Fig. 17.
20

13. A monoclonal antibody directed against an
epitope encoded in HCV cDNA, wherein the HCV cDNA is of a
sequence indicated by nucleotide numbers -319 to 1348 or
8659 to 8866 in Fig. 17, or is the sequence present in
25 clone 13i, or clone 26j, or clone 59a, or clone 84a, or
clone CA156e, or clone 167b, or clone pil4a, or clone
CA216a, or clone CA290a, or clone ag30a, or clone 205a, or
clone 18g, or clone 16jh.

30 14. A preparation of purified polyclonal anti-
bodies directed against a polypeptide comprised of an
epitope encoded within HCV cDNA, wherein the HCV cDNA is
of a sequence indicated by nucleotide numbers -319 to 1348
or 8659 to 8866 in Fig. 17, or is the sequence present in
35 clone 13i, or clone 26j, or clone 59a, or clone 84a, or
clone CA156e, or clone 167b, or clone pil4a, or clone

CA216a, or clone CA290a, or clone ag30a, or clone 205a, or clone 18g, or clone 16jh.

- 5 15. A polynucleotide probe for HCV, wherein the probe is comprised of an HCV sequence derived from an HCV cDNA sequence indicated by nucleotide numbers -319 to 1348 or 8659 to 8866 in Fig. 17, or from the complement of the HCV cDNA sequence.
- 10
16. A kit for analyzing samples for the presence of polynucleotides from HCV comprising a polynucleotide probe containing a nucleotide sequence of about 8 or more nucleotides, wherein the nucleotide
- 15 sequence is derived from HCV cDNA which is of a sequence indicated by nucleotide numbers -319 to 1348 or 8659 to 8866 in Fig. 17, wherein the polynucleotide probe is in a suitable container.
- 20
17. A kit for analyzing samples for the presence of an HCV antigen comprising an antibody which reacts immunologically with an HCV antigen, wherein the antigen contains an epitope encoded within HCV cDNA which is of a
- 25 sequence indicated by nucleotide numbers -319 to 1348 or 8659 to 8866 in Fig. 17, or wherein the HCV cDNA is in clone 13i, or clone 26j, or clone 59a, or clone 84a, or clone CA156e, or clone 167b, or clone p114a, or clone CA216a, or clone CA290a, or clone ag30a, or clone 205a, or clone 18g, or clone 16jh.
- 30
18. A kit for analyzing samples for the presence of an HCV antibody comprising an antigenic polypeptide containing an HCV epitope encoded within HCV cDNA which is of a sequence indicated by nucleotide
- 35 numbers -319 to 1348 or 8659 to 8866 in Fig. 17, or is in clone 13i, or clone 26j, or clone 59a, or clone 84a, or

clone CA156e, or clone 167b, or clone pil4a, or clone
CA216a, or clone CA290a, or clone ag30a, or clone 205a, or
clone 18g, or clone 16jh.

19. A kit for analyzing samples for the pres-
ence of an HCV antibody comprising an antigenic
polypeptide expressed from HCV cDNA in clone CA279a, or
clone CA74a, or clone 13i, or clone CA290a, or clone 33C
or clone 40b, or clone 33b, or clone 25c, or clone 14c, or
clone 8f, or clone 33f, or clone 33g, or clone 39c, or
clone 15e, wherein the antigenic polypeptide is present in
a suitable container.

20. A method for detecting HCV nucleic acids in
a sample comprising:

(a) reacting nucleic acids of the sample with a
polynucleotide probe for HCV, wherein the probe is
comprised of an HCV sequence derived from an HCV cDNA
sequence is of a sequence indicated by nucleotide numbers
-319 to 1348 or 8659 to 8866 in Fig. 17, and wherein the
reacting is under conditions which allow the formation of
a polynucleotide duplex between the probe and the HCV
nucleic acid from the sample,

(b) detecting a polynucleotide duplex which
contains the probe, formed in step (a).

21. An immunoassay for detecting an HCV antigen
comprising:

(a) incubating a sample suspected of containing
an HCV antigen with an antibody directed against an HCV
epitope encoded in HCV cDNA, wherein the HCV cDNA is of a
sequence indicated by nucleotide numbers -319 to 1348 or
8659 to 8866 in Fig. 17, or is the sequence present in
clone 13i, or clone 26j, or clone 59a, or clone 84a, or
clone CA156e, or clone 167b, or clone pil4a, or clone

CA216a, or clone CA290a, or clone ag30a, or clone 205a, or clone 18g, or clone 16jh, and wherein the incubating is under conditions which allow formation of an antigen-antibody complex; and (b) detecting an antibody-antigen complex formed in step (a) which contains the antibody.

22. An immunoassay for detecting antibodies directed against an HCV antigen comprising:

(a) incubating a sample suspected of containing anti-HCV antibodies with an antigen polypeptide containing an epitope encoded in HCV cDNA, wherein the HCV cDNA is of a sequence indicated by nucleotide numbers -319 to 1348 or 8659 to 8866 in Fig. 17, or is the sequence present in clone 13i, or clone 26j, or clone 59a, or clone 84a, or clone CA156e, or clone 167b, or clone pil4a, or clone CA216a, or clone CA290a, or clone ag30a, or clone 205a, or clone 18g, or clone 16jh, and wherein the incubating is under conditions which allow formation of an antigen-antibody complex; and

(b) detecting an antibody-antigen complex formed in step (a) which contains the antigen polypeptide.

23. An immunoassay for detecting antibodies directed against an HCV antigen comprising:

(a) incubating a sample suspected of containing anti-HCV antibodies with the polypeptide of claim 9, under conditions which allow formation of an antigen-antibody complex; and

(b) detecting an antibody-antigen complex formed in step (a) which contains the antigen polypeptide.

24. A vaccine for treatment of HCV infection comprising an immunogenic polypeptide containing an HCV epitope encoded in HCV cDNA, wherein the HCV cDNA is of a sequence indicated by nucleotide numbers -319 to 1348 or

8659 to 8866 in Fig. 17 or is the sequence present in clone 13i, or clone 26j, or clone 59a, or clone 84a, or clone CA156e, or clone 167b, or clone p114a, or clone CA216a, or clone CA290a, or clone ag30a, or clone 205a, or clone 18g, or clone 16jh, and wherein the immunogenic polypeptide is present in a pharmacologically effective dose in a pharmaceutically acceptable excipient.

25. A method for producing antibodies to HCV comprising administering to an individual an isolated immunogenic polypeptide containing an HCV epitope encoded in HCV cDNA, wherein the HCV cDNA is of a sequence indicated by nucleotide numbers -319 to 1348 or 8659 to 8866 in Fig. 17, or is of the sequence present in clone CA279a, or clone CA74a, or clone 13i, or clone CA290a, or clone 33C or clone 40b, or clone 33b, or clone 25c, or clone 14c, or clone 8f, or clone 33f, or clone 33g, or clone 39c, or clone 15e, and wherein the immunogenic polypeptide is present in a pharmacologically effective dose in a pharmaceutically acceptable excipient.

26. An antisense polynucleotide derived from HCV cDNA, wherein the HCV cDNA is that shown in Fig. 17.

27. A method for preparing purified fusion polypeptide C100-3 comprising:

- (a) providing a crude cell lysate containing polypeptide C100-3,
- (b) treating the crude cell lysate with an amount of acetone which causes the polypeptide to precipitate,
- (c) isolating and solubilizing the precipitated material,
- (d) isolating the C100-3 polypeptide by anion exchange chromatography, and

(e) further isolating the C100-3 polypeptide of step (d) by gel filtration.

5

28. A method for preparing an HCV polypeptide comprising:

(a) providing a host cell transformed with a recombinant expression system comprising an open reading frame (ORF) of DNA derived from HCV cDNA, wherein the HCV cDNA is in clone 13i, or clone 26j, or clone 59a, or clone 84a, or clone CA156e, or clone 167b, or clone pi14a, or clone CA216a, or clone CA290a, or clone ag30a, or clone 205a, or clone 18g, or clone 16jh, or wherein the HCV cDNA is of a sequence indicated by nucleotide numbers -319 to 1348 or 8659 to 8866 in Fig. 17, wherein the ORF is operably linked to a control sequence compatible with a desired host; and

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(b) incubating the host cell under conditions with allow expression of the HCV polypeptide.

29. A method for preparing an immunogenic HCV polypeptide comprising:

(a) providing a host cell transformed with a recombinant expression vector comprising an ORF of DNA derived from HCV cDNA, wherein the HCV cDNA is comprised of a sequence derived from the HCV cDNA sequence in clone CA279a, or clone CA74a, or clone 13i, or clone CA290a, or clone 33c, or clone 40b, or clone 33b, or clone 25c, or clone 14c, or clone 8f, or clone 33f, or clone 33g, or clone 39c, or clone 15e, wherein the OEF is operably linked to a control sequence compatible with the desired host; and

25

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(b) incubating the host cell under conditions which allow expression of the HCV polypeptide.

35

30. A method for preparing a host cell

transformed with a recombinant polynucleotide comprising a sequence of HCV cDNA derived from the HCV cDNA in clone 131, or clone 26j, or clone 59a, or clone 84a, or clone 5
CA156e, or clone 167b, or clone p114a, or clone CA216a, or clone CA290a, or clone ag30a, or clone 205a, or clone 18g, or clone 16jh, or wherein the HCV cDNA is of a sequence indicated by nucleotide numbers -319 to 1348 or 8659 to 8866 in Fig. 17 comprising:

- 10 (a) providing a host cell capable of transformation;
- (b) providing the recombinant polynucleotide;
- and
- (c) incubating (a) with (b) under conditions
15 which allow transformation of the host cell with the polynucleotide.

31. A method for preparing a recombinant polynucleotide comprised of a sequence of HCV cDNA derived from the HCV cDNA in clone 131, or clone 26j, or clone 59a, or clone 84a, or clone CA156e, or clone 167b, or clone p114a, or clone CA216a, or clone CA290a, or clone ag30a, or clone 205a, or clone 18g, or clone 16jh, or
20 wherein the HCV cDNA is of a sequence indicated by nucleotide numbers -319 to 1348 or 8659 to 8866 in Fig. 17 comprising:

- 25 (a) providing a host cell transformed with the recombinant polynucleotide; and
- (b) isolating said polynucleotide from said
30 host cell.

32. A method for preparing blood free of HCV comprising:

- 35 (a) providing a sample of blood suspected of containing HCV and anti-HCV antibodies;

- (b) providing an immunogenic polypeptide prepared according to claim 28 or 29;
- 5 (c) incubating the sample of (a) with the immunogenic polypeptide of (b) under conditions which allow the formation of antibody-HCV polypeptide complexes;
- (d) detecting the complexes formed in step (c); and
- 10 (e) saving the blood from which complexes were not detected in (d).

33. A method for preparing blood free of HCV comprising:

- 15 (a) providing nucleic acids from a sample of blood suspected of containing HCV polynucleotides;
- (b) providing a probe for HCV, wherein the probe is comprised of an HCV sequence derived from an HCV cDNA which is of a sequence indicated by nucleotide numbers -319 to 1348 or 8659 to 8866 in Fig. 17,
- 20 (c) reacting (a) with (b) under conditions which allow the formation of a polynucleotide duplex between the probe and the HCV nucleic acid from the sample;
- (d) detecting a polynucleotide which contains
- 25 the probe, formed in step (c); and
- (e) saving the blood from which complexes were not detected in (d).

34. A method for producing a hybridoma which produces anti-HCV monoclonal antibodies comprising:

- 30 (a) immunizing an individual with an immunogenic polypeptide containing an epitope encoded in HCV cDNA, wherein the HCV cDNA is HCV cDNA in clone 13i, or clone 26j, or clone 59a, or clone 84a, or clone CA156e,
- 35 or clone 167b, or clone p114a, or clone CA216a, or clone

CA290a, or clone ag30a, or clone 205a, or clone 18g, or
clone 16jh, or wherein the HCV cDNA is of a sequence
indicated by nucleotide numbers -319 to 1348 or 8659 to
5 8866 in Fig. 17; or

(b) immunizing an individual with an
immunogenic polypeptide prepared according to claim 29;

(c) immortalizing antibody producing cells from
the immunized individual;

10 (d) selecting an immortal cell which produces
antibodies which react with an HCV epitope in the
immunogenic polypeptide of (a) or (b); and

(e) growing said immortal cell.

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FIG. 1 Translation of DNA 12f

IlePheLysIleArgMetTyrValGlyGlyValGluHisArgLeuGluAlaAlaCysAsn
1 CCATATTTTAAATCAGGATGTACGTGGGAGGGGTCGAACACAGGCTGGAAGCTGCCTGCA
GGTATAAATTTTAGTCCTACATGCACCCTCCCCAGCTTGTGTCCGACCTTCGACGGACGT

TrpThrArgGlyGluArgCysAspLeuGluAspArgAspArgSerGluLeuSerProLeu
61 ACTGGACGCGGGGCGAACGTTGCGATCTGGAAGACAGGGACAGGTCCGAGCTCAGCCCGT
TGACCTGCGCCCCGCTTGCAACGCTAGACCTTCTGTCCCTGTCCAGGCTCGAGTCGGGCA

LeuLeuThrThrThrGlnTrpGlnValLeuProCysSerPheThrThrLeuProAlaLeu
121 TACTGCTGACCACTACACAGTGGCAGGTCTCCCGTGTTTCCTTCACAACCCTACCAGCCT
ATGACGACTGGTGATGTGTACCGTCCAGGAGGGCACAAGGAAGTGTGGGATGGTCCGA

SerThrGlyLeuIleHisLeuHisGlnAsnIleValAspValGlnTyrLeuTyrGlyVal
181 TGTCCACCGGCCCTCATCCACCTCCACCAGAACATTGTGGACGTGCAGTACTTGTACGGGG
ACAGGTGGCCGGAGTAGGTGGAGGTGGTCTTGTAACACCTGCACGTCATGAACATGCCCC

GlySerSerIleAlaSerTrpAlaIleLysTrpGluTyrValValLeuLeuPheLeuLeu
241 TGGGGTCAAGCATCGCGTCCTGGGCCATTAAGTGGGAGTACGTCGTTCTCCTGTTCTTC
ACCCAGTTCGTAGCGCAGGACCCGGTAATTCACCCTCATGCAGCAAGAGGACAAGGAAG

LeuAlaAspAlaArgValCysSerCysLeuTrpMetMetLeuLeuIleSerGlnAlaGlu
301 TGCTTGACAGACGCGCGCGTCTGCTCCTGCTTGTGGATGATGCTACTCATATCCCAAGCGG
ACGAACGTCTGCGCGCGCAGACGAGGACGAACACCTACTACGATGAGTATAGGGTTCGCC

AlaAlaLeuGluAsnLeuValIleLeuAsnAlaAlaSerLeuAlaGlyThrHisGlyLeu
361 AGGCGGCTTTGGAGAACCTCGTAATACTTAATGCAGCATCCCTGGCCGGGACGCACGGTC
TCCGCCGAAACCTCTTGGAGCATTATGAATTACGTCGTAGGGACCGGCCCTGCGTGCCAG

Val
421 TTGTATC
AACATAG

FIG. 2-1 Translation of DNA k9-1

1 GlyCysProGluArgLeuAlaSerCysArgProLeuThrAspPheAspGlnGlyTrpGly
CAGGCTGTCCTGAGAGGCTAGCCAGCTGCCGACCCCTTACCGATTTTGACCAGGGCTGGG
GTCCGACAGGACTCTCCGATCGGTTCGACGGCTGGGGAATGGCTAAACTGGTCCCGACCC

61 ProIleSerTyrAlaAsnGlySerGlyProAspGlnArgProTyrCysTrpHisTyrPro
GCCCTATCAGTTATGCCAACGGAAGCGGCCCGACCGCGCCCTACTGCTGGCACTACC
CGGGATAGTCAATACGGTTGCCCTTCGCCGGGGCTGGTCGCGGGGATGACGACCGTGATGG

121 ProLysProCysGlyIleValProAlaLysSerValCysGlyProValTyrCysPheThr
CCCCAAAACCTTGCGGTATTGTGCCCCGAAGAGTGTGTGGTCCGGTATATTGCTTCA
GGGGTTTTTGAACGCCATAACACGGGCGCTTCTCACACACACCAGGCCATATAACGAAGT

181 ProSerProValValValGlyThrThrAspArgSerGlyAlaProThrTyrSerTrpGly
CTCCCAGCCCCGTGGTGGTGGGAACGACCGACAGGTCGGGCGCGCCACCTACAGCTGGG
GAGGGTCGGGGCACCACCACCTTGCTGGCTGTCCAGCCCGCGGGGTGGATGTCGACCC

241 GluAsnAspThrAspValPheValLeuAsnAsnThrArgProProLeuGlyAsnTrpPhe
GTGAAATGATACGGACGCTCTTCGTCCTTAACAATACCAGGCCACCGCTGGGCAATTGGT
CACTTTTACTATGCCTGCAGAAGCAGGAATTGTTATGGTCCGGTGGCGACCCGTTAACCA

301 GlyCysThrTrpMetAsnSerThrGlyPheThrLysValCysGlyAlaProProCysVal
TCGGTTGTACCTGGATGAACCTCACTGATTACCAAAGTGTGCGGAGCGCCTCCTTGTC
AGCCAACATGGACCTACTTGAGTTGACCTAAGTGGTTTCACACGCCTCGCGGAGGAACAC

361 IleGlyGlyAlaGlyAsnAsnThrLeuHisCysProThrAspCysPheArgLysHisPro
TCATCGGAGGGGCGGGCAACAACACCCTGCACTGCCCCACTGATTGCTTCCGCAAGCATC
AGTAGCCTCCCCGCGCGTTGTTGTGGGACGTGACGGGGTGACTAACGAAGCGTTCTGTAG

421 AspAlaThrTyrSerArgCysGlySerGlyProTrpIleThrProArgCysLeuValAsp
CGGACGCCACATACTCTCGGTGCGGCTCCGGTCCCTGGATCACACCCAGGTGCCTGGTCG
GCCTGCGGTGTATGAGAGCCACGCCGAGGCCAGGGACCTAGTGTGGGTCCACGGACCAGC

481 TyrProTyrArgLeuTrpHisTyrProCysThrIleAsnTyrThrIlePheLysIleArg
ACTACCCGTATAGGCTTTGGCATTATCCTTGTACCATCACTACACTATATTTAAATCA
TGATGGGCATATCCGAAACCGTAATAGGAACATGGTAGTTGATGTGATATAAATTTTAGT

541 MetTyrValGlyGlyValGluHisArgLeuGluAlaAlaCysAsnTrpThrArgGlyGlu
GGATGTACGTGGGAGGGGTCGAGCACAGGCTGGAAGCTGCCTGCAACTGGACGCGGGGCG
CCTACATGCACCCTCCCCAGCTCGTGTCCGACCTTCGACGGACGTTGACCTGCGCCCCGC

601 ArgCysAspLeuGluAspArgAspArgSerGluLeuSerProLeuLeuLeuThrThrThr
AACGTTGCGATCTGGAAGATAGGGACAGGTCCGAGCTCAGCCCGTTACTGCTGACCACTA
TTGCAACGCTAGACCTTCTATCCCTGTCCAGGCTCGAGTCGGGCAATGACGACTGGTGTAT

661 GlnTrpGlnValLeuProCysSerPheThrThrLeuProAlaLeuSerThrGlyLeuIle
CACAGTGGCAGGTCCTCCCGTGTTCCTTCAACCCCTGCCAGCCTTGTCCACCGGCCTCA
GTGTCACCGTCCAGGAGGGCACAAGGAAGTGTGGGACGGTCGGAACAGGTGGCCGGAGT

721 HisLeuHisGlnAsnIleValAspValGlnTyrLeuTyrGlyValGlySerSerIleAla
TCCACCTCCACCAGAACATTGTGGACGTGCAGTACTTGTACGGGGTGGGGTCAAGCATCG
AGGTGGAGGTGGTCTGTAAACACCTGCACGTCATGAACATGCCCCACCCAGTTCGTAGC

781 SerTrpAlaIleLysTrpGluTyrValValLeuLeuPheLeuLeuAlaAspAlaArg
CGTCCTGGGCCATTAAGTGGGAGTACGTCGTCCTCCTGTTCTTCTGCTTGCAGACGCGC
GCAGGACCCGGTAATTCACCCTCATGCAGCAGGAGGACAAGGAAGACGAACGTCGCGCG

ValCysSerCysLeuTrpMetMetLeuLeuIleSerGlnAlaGluAlaAlaLeuGluAsn
841 GCGTCTGCTCCTGCTTGTGGATGATGCTACTCATATCCCAAGCGGAAGCGGCTTTGGAGA
CGCAGACGAGGACGAACACCTACTACGATGAGTATAGGGTTCGCCCTTCGCCGAAACCTCT

LeuValIleLeuAsnAlaAlaSerLeuAlaGlyThrHisGlyLeuValSerPheLeuVal
901 ACCTCGTAATACTTAATGCAGCATCCCTGGCCGGGACGCACGGTCTTGTATCCTTCTCTCG
TGGAGCATTATGAATTACGTCGTAGGGACCGGCCCTGCGTGCCAGAACATAGGAAGGAGC

PhePheCysPheAlaTrpTyrLeuLysGlyLysTrpValProGlyAlaValTyrThrPhe
961 TGTTCCTTGCTTTGCATGGTATCTGAAGGGTAAGTGGGTGCCCGGAGCGGTCTACACCT
ACAAGAAGACGAAACGTACCATAGACTTCCCATTCACCCACGGGCCTCGCCAGATGTGGA

TyrGlyMetTrpProLeuLeuLeuLeuLeuAlaLeuProGlnArgAlaTyrAlaLeu
1021 TCTACGGGATGTGGCCTCTCCTCCTGCTCCTGTTGGCGTTGCCCGAGCGGGCGTACGCGC
AGATGCCCTACACCGGAGAGGAGGACGAGGACAACCGCAACGGGGTTCGCCCGCATGCGCG

AspThrGluValAlaAlaSerCysGlyGlyValValLeuValGlyLeuMetAlaLeuThr
1081 TGGACACGGAGGTGGCCGCGTCGTGTGGCGGTGTTGTTCTCGTCGGGTTGATGGCGCTAA
ACCTGTGCCTCCACCGGCGCAGCACACCGCCACAACAAGAGCAGCCCAACTACCGCGATT

LeuSerProTyrTyrLysArgTyrIleSerTrpCysLeuTrpTrpLeuGlnTyrPheLeu
1141 CTCTGTCAACATATTACAAGCGCTATATCAGCTGGTGCTTGTGGTGGCTTCAGTATTTTC
GAGACAGTGGTATAATGTTTCGCGATATAGTCGACCACGAACACCACCGAAGTCATAAAAG

ThrArgValGluAlaGlnLeuHisValTrpIleProProLeuAsnValArgGlyGlyArg
1201 TGACCAGAGTGGAAGCGCAACTGCACGTGTGGATTCCCCCCTCAACGTCCGAGGGGGGC
ACTGGTCTACCTTCGCGTTGACGTGCACACCTAAGGGGGGAGTTGCAGGCTCCCCCG

AspAlaValIleLeuLeuMetCysAlaValHisProThrLeuValPheAspIleThrLys
1261 GCGACGCTGTCTTACTCATGTGTGCTGTACACCGACTCTGGTATTTGACATCACCA
CGCTGCGACAGTAGAATGAGTACACACGACATGTGGGCTGAGACCATAAACTGTAGTGGT

LeuLeuLeuAlaValPheGlyProLeuTrpIleLeuGlnAla
1321 AATTGCTGCTGGCCGTCTTCGGACCCCTTTGGATTCTTCAAGCCAG
TTAACGACGACCGGCAGAAGCCTGGGGAAACCTAAGAAGTTCGGTC

FIG. 2-2

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FIG. 3

1 GlyAlaGlyLysArgValTyrTyrLeuThrArgAspProThrThrProLeuAlaArgAla
 CGGCGCTGGAAGAGGGTCTACTACCTCACCCGTGACCCTACAACCCCTCGCGAGAGC
 GCCGCGACCTTTCTCCCAGATGATGGAGTGGGCACTGGGATGTTGGGGGGAGCGCTCTCG

 61 AlaTrpGluThrAlaArgHisThrProValAsnSerTrpLeuGlyAsnIleIleMetPhe
 TCGTGGGAGACAGCAAGACACACTCCAGTCAATTCCTGGCTAGGCAACATAATCATGTT
 ACGCACCTCTGTCTGTTCTGTGTGAGGTCAGTTAAGGACCGATCCGTTGTATTAGTACAA

 121 AlaProThrLeuTrpAlaArgMetIleLeuMetThrHisPhePheSerValLeuIleAla
 TGCCCCCACTGTGGGCGAGGATGATACTGATGACCCATTCTTTAGCGTCCTTATAGC
 ACGGGGGTGTGACACCCGCTCCTACTATGACTACTGGGTAAAGAAATCGCAGGAATATCG

 181 ArgAspGlnLeuGluGlnAlaLeuAspCysGluIleTyrGlyAlaCysTyrSerIleGlu
 CAGGGACAGCTTGAACAGGCCCTCGATTGCGAGATCTACGGGGCCTGCTACTCCATAGA
 GTCCCTGGTCAACTTGTCCGGGAGCTAACGCTCTAGATGCCCCGACGATGAGGTATCT

 241 ProLeuAspLeuProProIleIleGlnArgLeu
 ACCACTTGATCTACCTCCAATCATTCAAAGACTC
 TGGTGAAGTAGATGGAGGTTAGTAAGTTTCTGAG

FIG. 5

Translation of DNA 26j

1 LeuPheTyrHisHisLysPheAsnSerSerGlyCysProGluArgLeuAlaSerCysArg
 GCTTTTCTATCACCACAAGTTCAACTCTTCAGGCTGTCCTGAGAGGCTAGCCAGCTGCCG
 CGAAAAGATAGTGGTGTTCAGTTGAGAAGTCCGACAGGACTCTCCGATCGGTCGACGGC

 61 ProLeuThrAspPheAspGlnGlyTrpGlyProIleSerTyrAlaAsnGlySerGlyPro
 ACCCCTTACCGATTTTGACCAGGGCTGGGGCCCTATCAGTTATGCCAACGGAAGCGGCCC
 TGGGGAATGGCTAAAACTGGTCCCGACCCGGGATAGTCAATACGGTTGCCTTCGCCGGG

 121 AspGlnArgProTyrCysTrpHisTyrProProLysProCysGlyIleValProAlaLys
 CGACCAGCGCCCCTACTGCTGGCACTACCCCCAAAACCTTGCGGTATTGTGCCCCGAA
 GCTGGTCGCGGGGATGACGACCGTGATGGGGGGTTTTGGAACGCCATAACACGGGCGCTT

 181 SerValCysGlyProValTyrCysPheThrProSerProValValVal
 GAGTGTGTGTGGTCCGGTATATTGCTTCACTCCAGCCCCGTGGTGGTGGG
 CTCACACACACCAGGCCATATAACGAAGTGAGGGTCGGGGCACCACCACC

---Overlap with 131---

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FIG. 4

Translation of DNA 13i

ProSerProValValValGlyThrThrAspArgSerGlyAlaProThrTyrSerTrpGly
1 CTCCCAGCCCCGTGGTGGTGGGAACGACCGACAGGTCGGGCGCGCCTACCTACAGCTGGG
GAGGGTCGGGGCACCACCACCCTTGCTGGCTGTCCAGCCCGCGCGGATGGATGTCGACCC

GluAsnAspThrAspValPheValLeuAsnAsnThrArgProProLeuGlyAsnTrpPhe
61 GTGAAAATGATACGGACGTCTTCGTCCTTAACAATACCAGGCCACCGCTGGGCAATTGGT
CACTTTTACTATGCCTGCAGAAGCAGGAATTGTTATGGTCCGGTGGCGACCCGTTAACCA

GlyCysThrTrpMetAsnSerThrGlyPheThrLysValCysGlyAlaProProCysVal
121 TCGGTTGTACCTGGATGAACTCAACTGGATTACCAAAGTGTGCGGAGCGCCTCCTTGTG
AGCCAACATGGACCTACTTGAGTTGACCTAAGTGGTTTCACACGCCCTCGCGGAGGAACAC

IleGlyGlyAlaGlyAsnAsnThrLeuHisCysProThrAspCysPheArgLysHisPro
181 TCATCGGAGGGGCGGGCAACAACACCCTGCACTGCCCCACTGATTGCTTCCGCAAGCATC
AGTAGCCTCCCCGCCCGTTGTTGTGGGACGTGACGGGGTGAATAACGAAGCGTTCTGTAG

AspAlaThrTyrSerArgCysGlySerGlyProTrpLeuThrProArgCysLeuValAsp
241 CGGACGCCACATACTCTCGGTGCGGCTCCGGTCCCTGGCTCACACCCAGGTGCCTGGTCTG
GCCTGCGGTGTATGAGAGCCACGCCGAGGCCAGGGACCGAGTGTGGGTCCACGGACCAGC

TyrProTyrArgLeuTrpHisTyrProCysThrIleAsnTyrThrIlePheLysIleArg
301 ACTACCCGTATAGGCTTTGGCATTATCCTTGTACCATCAACTACACCATATTTAAATCA
TGATGGGCATATCCGAAACCGTAATAGGAACATGGTAGTTGATGTGGTATAAATTTTAGT

MetTyrValGlyGlyValGluHisArgLeuGluAlaAlaCysAsnTrpThrArgGlyGlu
361 GGATGTACGTGGGAGGGGTCGAGCACAGGCTGGAAGCTGCCTGCAACTGGACGCGGGGCG
CCTACATGCACCCTCCCCAGCTCGTGTCCGACCTTCGACGGACGTTGACCTGCGCCCCGC

-----Overlap with 12f-----
ArgCysAspLeuGluAspArgAspArgSerGluLeuSerProLeuLeuLeuThrThrThr
421 AACGTTGCGATCTGGAAGACAGGGACAGGTCCGAGCTCAGCCCGTTACTGCTGACCACTA
TTGCAACGCTAGACCTTCTGTCCCTGTCCAGGCTCGAGTCGGGCAATGACGACTGGTGTAT

GlnTrpGlnValLeuProCysSerPheThrThrLeuProAlaLeuSerThrGlyLeu
481 CACAGTGGCAGGTCCTCCCGTGTTCCTTCACAACCCTGCCAGCCTTGTCCACCGGCCTCA
GTGTCACCGTCCAGGAGGGCACAAGGAAGTGTGGGACGGTCGGAACAGGTGGCCGGAGT

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FIG. 6

Translation of DNA CA59a

LeuValMetAlaGlnLeuLeuArgIleProGlnAlaIleLeuAspMetIleAlaGlyAla
1 TTGGTAATGGCTCAGCTGCTCCGGATCCCACAAGCCATCTTGGACATGATCGCTGGTGCT
AACCATTACCGAGTCGACGAGGCCTAGGGTGTTCGGTAGAACCTGTACTAGCGACCACGA

HisTrpGlyValLeuAlaGlyIleAlaTyrPheSerMetValGlyAsnTrpAlaLysVal
61 CACTGGGGAGTCCTGGCGGGCATAGCGTATTTCTCCATGGTGGGGAAGTGGGCGAAGGTC
GTGACCCCTCAGGACCGCCCGTATCGCATAAAGAGGTACCACCCCTTGACCCGCTTCCAG

LeuValValLeuLeuLeuPheAlaGlyValAspAlaGluThrHisValThrGlyGlySer
121 CTGGTAGTGCTGCTGCTATTTGCCGGCGTCGACGCGGAAACCCACGTCACCGGGGGAAGT
GACCATCAGACGACGATAAACGGCCGCGAGCTGCGCCTTGGGTGCAGTGGCCCCCTTCA

AlaGlyHisThrValSerGlyPheValSerLeuLeuAlaProGlyAlaLysGlnAsnVal
181 GCCGGCCACACTGTGTCTGGATTTGTTAGCCTCCTCGCACCAGGCGCCAAGCAGAACGTC
CGGCCGGTGTGACACAGACCTAAACAATCGGAGGAGCGTGGTCCGCGGTTTCGTCTTGACG

GlnLeuIleAsnThrAsnGlySerTrpHisLeuAsnSerThrAlaLeuAsnCysAsnAsp
241 CAGCTGATCAACACCAACGGCAGTTGGCACCTCAATAGCACGGCCCTGAACTGCAATGAT
GTCGACTAGTTGTGGTTGCCGTCAACCGTGGAGTTATCGTGCCGGGACTTGACGTTACTA

SerLeuAsnThrGlyTrpLeuAlaGlyLeuPheTyrHisHisLysPheAsnSerSerGly
301 AGCCTCAACACCGGCTGGTTGGCAGGGCTTTTCTATCACCACAAGTTCAACTCTTCAGGC
TCGGAGTTGTGGCCGACCAACCGTCCCGAAAAGATAGTGGTGTTCAGTTGAGAAGTCCG
-----Overlap with 26j-----

-----Overlap with K9-1-----
CysProGluArgLeuAlaSerCysArgPro
361 TGTCTGAGAGGCTAGCCAGCTGCCGACCCC
ACAGGACTCTCCGATCGGTCGACGGCTGGGG

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Translation of DNA CA84a

FIG. 7

1 GlnGlyCysAsnCysSerIleTyrProGlyHisIleThrGlyHisArgMetAlaTrpAsp
 CGCAAGGTTGCAATTGCTCTATCTATCCCCGCCATATAACGGGTCACCGCATGGCATGGG
 GCGTTCCAACGTTAACGAGATAGATAGGGCCGGTATATTGCCAGTGCGGTACCGTACCC

 61 MetMetMetAsnTrpSerProThrThrAlaLeuValMetAlaGlnLeuLeuArgIlePro
 ATATGATGATGAACTGGTCCCCCTACGACGGCGTTGGTAATGGCTCAGCTGCTCCGGATCC
 TATACTACTACTTGACCAGGGGATGCTGCCGCAACCATTACCGAGTCGACGAGGCCTAGG

 121 GlnAlaIleLeuAspMetIleAlaGlyAlaHisTrpGlyValLeuAlaGlyIleAlaTyr
 CACAAGCCATCTTGGACATGATCGCTGGTGCTCACTGGGGAGTCTTGGCGGGCATAGCGT
 GTGTTCCGGTAGAACCTGTACTAGCGACCACGAGTGACCCCTCAGGACCGCCCGTATCGCA

 -----Overlap with CA59a-----
 181 PheSerMetValGlyAsnTrpAlaLysValLeuValValLeuLeuLeuPheAlaGlyVal
 ATTTCTCCATGGTGGGGAAGTGGCGAAGGTCCTGGTAGTGCTGCTGCTATTTGCCGGCG
 TAAAGAGGTACCACCCCTTGACCCGCTTCCAGGACCATCAGACGACGATAAACGGCCCG

 241 AspAlaGluThrHisValThrGly
 TCGACGCGGAAACCCACGTCACCGGGG
 AGCTGCGCCTTTGGGTGCAGTGGCCCC

Translation of DNA CA156e

FIG. 8

1 CysTrpValAlaMetThrProThrValAlaThrArgAspGlyLysLeuProAlaThrGln
 GTGTTGGGTGGCGATGACCCCTACGGTGGCCACCAGGGATGGCAAACCTCCCCGCGACGCA
 CACAACCCACCGCTACTGGGGATGCCACCGTGGTCCCTACCGTTTGAGGGGCGCTGCGT
 61 LeuArgArgHisIleAspLeuLeuValGlySerAlaThrLeuCysSerAlaLeuTyrVal
 GCTTCGACGTCACATCGATCTGCTTGTGCGGAGCGCCACCCTCTGTTCCGCCCTCTACGT
 CGAAGCTGCAGTGTAGCTAGACGAACAGCCCTCGCGGTGGGAGACAAGCCGGGAGATGCA
 121 GlyAspLeuCysGlySerValPheLeuValGlyGlnLeuPheThrPheSerProArgArg
 GGGGACCTATGCGGGTCTGTCTTTCTTGTGCGGCAACTGTTACCTTCTCTCCAGGCG
 CCCCCTGGATACGCCAGACAGAAAGAACAGCCGGTTGACAAGTGAAGAGAGGGTCCGC

 181 HisTrpThrThrGlnGlyCysAsnCysSerIleTyrProGlyHisIleThrGlyHisArg
 CCACTGGACGACGCAAGGTTGCAATTGCTCTATCTATCCCCGCCATATAACGGGTCACCG
 GGTGACCTGCTGCGTTCCAACGTTAACGAGATAGATAGGGCCGGTATATTGCCAGTGCG

 -----Overlap with CA84a-----
 241 MetAlaTrpAspMetMetMetAsnTrpSerProThrThrAlaLeuValValAlaGlnLeu
 CATGGCATGGGATATGATGATGAACTGGTCCCCCTACGACGGCGTTGGTAGTGCTCAGCT
 GTACCGTACCCCTATACTACTTACCAGGGGATGCTGCCGCAACCATCACCAGTCTGA

 301 LeuArgIleProGlnAla
 GCTCCGGATCCCACAAGCC
 CGAGGCCTAGGGTGTTCCG

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FIG. 9

Translation of DNA CA167b

1 SerThrGlyLeuTyrHisValThrAsnAspCysProAsnSerSerIleValTyrGluAla
CTCCACGGGGCTTTACCACGTCACCAATGATTGCCCTAACTCGAGTATTGTGTACGAGGC
GAGGTGCCCCGAAATGGTGCAGTGGTTACTAACGGGATTGAGCTCATAACACATGCTCCG

61 AlaAspAlaIleLeuHisThrProGlyCysValProCysValArgGluGlyAsnAlaSer
GGCCGATGCCATCCTGCACACTCCGGGGTGGTCCCTTGCGTTTCGTGAGGGCAACGCCTC
CCGGCTACGGTAGGACGTGTGAGGCCCCACGCAGGGAACGCAAGCACTCCCGTTGCGGAG

121 ArgCysTrpValAlaMetThrProThrValAlaThrArgAspGlyLysLeuProAlaThr
GAGGTGTTGGGTGGCGATGACCCCTACGGTGGCCACCAGGGATGGCAAACCTCCCCGCGAC
CTCCACAACCCACCGCTACTGGGGATGCCACCGGTGGTCCCTACCGTTTGAGGGGCGCTG

-----Overlap with CA156e-----

181 GlnLeuArgArgHisIleAspLeuLeuValGlySerAlaThrLeuCysSerAlaLeuTyr
GCAGCTTCGACGTCACATCGATCTGCTTGTGCGGAGCGCTACCCTCTGTTTCGGCCCTCTA
CGTCGAAGCTGCAGTGTAGCTAGACGAACAGCCCTCGCGATGGGAGACAAGCCGGGAGAT

241 ValGlyAspLeuCysGlySerValPheLeu
CGTGGGGGACTTGTGCGGGTCTGTCTTTCTTG
GCACCCCTGAACACGCCCAGACAGAAAGAAC

FIG. 10

Translation of DNA ssCA216a

1 ArgArgArgSerArgAsnLeuGlyLysValIleAspThrLeuThrCysGlyPheAlaAsp
CCCGGCGTAGGTTCGCGCAATTTGGGTAAGGTCATCGATACCCTTACGTGCGGCTTCGCCC
GGGCGCGCATCCAGCGCGTTAAACCCATTCCAGTAGCTATGGGAATGCACGCCGAAGCGGC

61 LeuMetGlyTyrIleProLeuValGlyAlaProLeuGlyGlyAlaAlaArgAlaLeuAla
ACCTCATGGGGTACATACCGCTCGTCGGCGCCCCCTCTTGGAGGCGCTGCCAGGGCCCTGG
TGGAGTACCCCATGTATGGCGAGCAGCCGCGGGGAGAACCTCCGCGACGGTCCCGGGACC

121 HisGlyValArgValLeuGluAspGlyValAsnTyrAlaThrGlyAsnLeuProGlyCys
CGCATGGCGTCCGGGTTCGGGAAGACGGCGTGAACATATGCAACAGGGAACCTTCCTGGTT
CGGTACCGCAGGCCCAAGACCTTCGCGCACCTTGATACGTTGTCCCTTGAAGGACCAA

181 SerPheSerIlePheLeuLeuAlaLeuLeuSerCysLeuThrValProAlaSerAlaTyr
GCTCTTTCTCTATCTTCCTTCTGGCCCTGCTCTCTTGCTTGACTGTGCCCGCTTCGGCCT
CGAGAAAGAGATAGAAGGAAGACCGGGACGAGAGAACGAACGACACGGGCGAAGCCGGA

241 GlnValArgAsnSerThrGlyLeuTyrHisValThrAsnAspCysProAsnSerSerIle
ACCAAGTGCGCAACTCCACGGGGCTTTACCACGTCACCAATGATTGCCCTAACTCGAGTA
TGGTTCACGCGTTGAGGTGCCCCGAAATGGTGACGTGGTTACTAACGGGATTGAGCTCAT

301 ValTyrGluAlaAlaAspAlaIleLeuHisThrProGlyCysValProCysValArgGlu
TTGTGTACGAAGCGGCCGATGCCATCCTGCACACTCCGGGGTGCGTCCCTTGCCTTCGTG
AACACATGCTTCGCCGGCTACGGTAGGACGTGTGAGGCCCCACGCAGGGAACGCAAGCAC

361 GlyAsnAlaSerArgCysTrpValAlaMetThrProThrValAla
AGGGCAACGCCTCGAGGTGTTGGGTGGCGATGACCCCTACGGTGGCC
TCCCGTTGCGGAGCTCCACAACCCACCGCTACTGGGGATGCCACCGG

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FIG. 11

Translation of DNA ssCA290a

LysLysAsnLysArgAsnThrAsnArgArgProGlnAspValLysPheProGlyGlyGly
1 AAAAAAAAAACAAACGTAACACCAACCGTCGCCCACAGGACGTCAAGTTCCCGGGTGGCG
TTTTTTTTTTTGTTCATTGTGGTTGGCAGCGGGTGTCTGCAGTTCAAGGGCCACCGC

GlnIleValGlyGlyValTyrLeuLeuProArgArgGlyProArgLeuGlyValArgAla
61 GTCAGATCGTTGGTGGAGTTTACTTGTGCGCGCAGGGGCCCTAGATTGGGTGTGCGCG
CAGTCTAGCAACCACCTCAAATGAACAACGGCGCGTCCCCGGGATCTAACCACACGCGC

ThrArgLysThrSerGluArgSerGlnProArgGlyArgArgGlnProIleProLysAla
121 CGACGAGAAAGACTTCCGAGCGGTGCGAACCTCGAGGTAGACGCCAGCCTATCCCCAAGG
GCTGCTCTTTCTGAAGGCTCGCCAGCGTTGGAGCTCCATCTGCGGTCCGATAGGGGTTC

ArgArgProGluGlyArgThrTrpAlaGlnProGlyTyrProTrpProLeuTyrGlyAsn
181 CTCGTCGGCCCGAGGGCAGGACCTGGGCTCAGCCCGGGTACCCCTGGCCCCCTCTATGGCA
GAGCAGCCGGGCTCCCGTCTGGACCCGAGTCGGGCCCCATGGGAACCGGGGAGATACCGT

GluGlyCysGlyTrpAlaGlyTrpLeuLeuSerProArgGlySerArgProSerTrpGly
241 ATGAGGGCTGCGGGTGGGCGGGATGGCTCCTGTCTCCCCGTGGCTCTCGGCCTAGCTGGG
TACTCCCGACGCCACCCGCCCTACCGAGGACAGAGGGGCACCGAGAGCCGGATCGACCC

ProThrAspProArgArgArgSerArgAsnLeuGlyLysValIleAspThrLeuThrCys
301 GCCCCACAGACCCCGCGTAGGTGCGCAATTTGGGTAAAGTCATCGATACCCTTACGT
CGGGGTGTCTGGGGGCCGCATCCAGCGGTTAAACCCATTCCAGTAGCTATGGGAATGCA

GlyPheAlaAspLeuMetGlyTyrIleProLeuValGlyAlaProLeuGlyGlyAlaAla
361 GCGGCTTCGCCGACCTCATGGGGTACATACCGCTCGTCGGCGCCCCCTCTTGGAGGCGCTG
CGCCGAAGCGGCTGGAGTACCCCATGTATGGCGAGCAGCCGCGGGGAGAACCTCCGCGAC

-----overlap with CA216a-----
ArgAlaLeuAlaHisGlyValArgValLeuGluAspGlyValAsnTyrAlaThrGlyAsn
421 CCAGGGCCCTGGCGCATGGCGTCCGGGTCTGGAAGACGGCGTGAACCTATGCAACAGGGA
GGTCCCCGGGACCGGTACCGCAGGCCCAAGACCTTCTGCCGCACTTGATACGTTGTCCCT

LeuProGlyCysSerPheSerThrPhe
481 ACCTTCCTGGTTGCTCTTTCTCTACCTTC
TGGAAGGACCAACGAGAAAGAGATGGAAG

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Translation of DNA ag30a

FIG. 12-1

#MetSerValValGlnProProGlyProProLeu

#MetAlaLeuValOP

1 CGCAGAAAGCGTCTAGCCATGGCGTTAGTATGAGTGTCTGTCAGCCTCCAGGACCCCCC
GCGTCTTTTCGCAGATCGGTACCGCAATCATACTCACAGCACGTCCGAGGTCTCGGGGGG

ProGlyGluProAM

61 TCCCGGGAGAGCCATAGTGGTCTGCGGAACCGGTGAGTACACCGGAATTGCCAGGACGAC
AGGGCCCTCTCGGTATCACCAGACGCCTTGGCCACTCATGTGGCCTTAACGGTCTGCTG

#MetProGlyAspLeuGlyValProProGlnAsp

121 CGGGTCCTTTCTTGGATCAACCGCTCAATGCCTGGAGATTGGGCGTGCCCCCGCAAGA
GCCAGGAAAGAACCTAGTTGGGCGAGTTACGGACCTCTAAACCCGCACGGGGGCGTTCT

CysAM

OP AM GlyAlaCys

*

181 CTGCTAGCCGAGTAGTGTGGGTGCGGAAAGGCCTTGTGGTACTGCCTGATAGGGTGCTT
GACGATCGGCTCATCACAACCCAGCGCTTTCGGAACACCATGACGGACTATCCACGAA

GluCysProGlyArgSerArgArgProCysThrMetSerThrAsnProLysProGlnLys

241 GCGAGTGCCCCGGGAGGTCTCGTAGACCGTGCACCATGAGCACGAATCCTAAACCTCAA
CGCTCACGGGGCCCTCCAGAGCATCTGGCACGTGGTACTCGTGCTTAGGATTGGAGTTT

LysAsnLysArgAsnThrAsnArgArgProGlnAspValLysPheProGlyGlyGlyGln

301 AAAAAACAAACGTAACACCAACCGTCGCCCACAGGACGTCAAGTTCCCGGGTGGCGGTC
TTTTTTTGTTCGATTGTGGTTGGCAGCGGTGTCCTGCAGTTCAAGGGCCACCGCCAG

IleValGlyGlyValTyrLeuLeuProArgArgGlyProArgLeuGlyValArgAlaThr

361 AGATCGTTGGTGGAGTTTACTTGTGTCGCGCAGGGGCCCTAGATTGGGTGTGCGCGCA
TCTAGCAACCACCTCAAATGAACAACGGCGCGTCCCCGGGATCTAACCACACGCGCGCT

ArgLysThrSerGluArgSerGlnProArgGlyArgArgGlnProIleProLysAlaArg

421 CGAGAAAGACTTCCGAGCGGTCGCAACCTCGAGGTAGACGTCAGCCTATCCCCAAGGCTC
GCTCTTTCTGAAGGCTCGCCAGCGTTGGAGCTCCATCTGCAGTCGGATAGGGGTTCGAG

ArgProGluGlyArgThrTrpAlaGlnProGlyTyrProTrpProLeuTyrGlyAsnGlu

481 -----overlap with CA290a-----
GTCGGCCCCGAGGGCAGGACCTGGGCTCAGCCCGGTACCCTTGGCCCCCTCTATGGCAATG
CAGCCGGGCTCCCGTCCGACCCGAGTCGGGGCCATGGGAACCGGGGAGATACCGTTAC

GlyCysGlyTrpAlaGlyTrpLeuLeuSerProArgGlySerArgProSerTrpGlyPro

541 AGGGCTGCGGGTGGGCGGGATGGCTCCTGTCTCCCCGTGGCTCTCGGCCTAGCTGGGGCC
TCCCGACGCCACCCGCCCTACCGAGGACAGAGGGGCACCGAGAGCCGGATCGACCCCGG

ThrAspProArgArgArgSerArgAsnLeuGlyLysValIleAspThrLeuThrCysGly

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601 CCACAGACCCCCGGCGTAGGTCGCGCAATTTGGGTAAGGTCATCGATAACCCTTACGTGCG
GGTGTCTGGGGGCCGCATCCAGCGCGTTAAACCCATTCCAGTAGCTATGGGAATGCACGC

Phe

661 GCTTC
CGAAG

- * - Start of long HCV ORF
- | - Putative first amino acid of large HCV polyprotein
- # - Putative small encoded peptides (that may play a translational regulatory role)

FIG. 12-2

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FIG. 13

Translation of DNA CA205a

ValLeuGlyArgGluArgProCysGlyThrAlaOP AM GlyAlaCysGluCysProGly
1 GTCTTGGGTGCGGAAAGGCCTTGTGGTACTGCCTGATAGGGTGCTTGCGAGTGCCCCGGG
CAGAACCAGCGCTTTCGGAACACCATGACGGACTATCCCACGAACGCTCACGGGGCCC

*

ArgSerArgArgProCysThrMetSerThrAsnProLysProGlnArgLysThrLysArg
61 AGGTCTCGTAGACCGTGCACCATGAGCACGAATCCTAAACCTCAAAGAAAAACAAACGT
TCCAGAGCATCTGGCACGTGGTACTCGTCTTAGGATTGGAGTTTCTTTTGGTTTGA

AsnThrAsnArgArgProGlnAspValLysPheProGlyGlyGlyGlnIleValGlyGly
121 AACACCAACCGTCGCCCACAGGACGTCAAGTTCCCGGGTGGCGGTGAGATCGTTGGTGA
TTGTGGTTGGCAGCGGGTGTCTGCAGTTCAAGGGCCCACCGCCAGTCTAGCAACCACCT

ValTyrLeuLeuProArgArgGlyProArgLeuGlyValArgAlaThrArgLysThrSer
181 GTTTACTTGTGCGCGCAGGGGCCCTAGATTGGGTGTGCGCGCGACGAGAAAGACTTCC
CAAATGAACAACGGCGCGTCCCCGGGATCTAACCACACGCGCGCTGCTCTTTCTGAAGG

-----overlap with CA290a-----

GluArgSerGlnProArgGlyArgArgGlnProIleProLysAlaArgArgProGluGly
241 GAGCGGTGCGCAACCTCGAGGTAGACGTCAGCCTATCCCCAAGGCTCGTCGGCCCGAGGGC
CTCGCCAGCGTTGGAGCTCCATCTGCAGTCGGATAGGGGTTCGAGCAGCCGGGCTCCCG

ArgThrTrpAlaGlnProGlyTyrProTrpProLeuTyrGlyAsnGluGlyCys
301 AGGACCTGGGCTCAGCCCGGGTACCCCTGGCCCTCTATGGCAATGAGGGCTGCG
TCCTGGACCCGAGTCGGGCCCATGGGAACCGGGGAGATACCGTTACTCCCGACGC

* = putative initiator methionine codon

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FIG. 14

Translation of DNA 18g

```

#ProProOP
#SerThrMetAsnHisSerProValArgAsnTyrCysLeuHisAlaGluSerValAM Pro
#LeuHisHisGluSerLeuProCysGluGluLeuLeuSerSerArgArgLysArgLeuAla
1 CTCCACCATGAATCACTCCCCTGTGAGGAACTACTGTCTTCACGCAGAAAGCGTCTAGCC
  GAGGTGGTACTTAGTGAGGGGACACTCCTTGATGACAGAAGTCCGTCTTTCGCAGATCGG

-----
#MetSerValValGlnProProGlyProProLeuProGlyGluProAM
MetAlaLeuValOP
61 ATGGCGTTAGTATGAGTGTGTCGTGCAGCCTCCAGGACCCCCCTCCCGGGAGAGCCATAGT
  TACCGCAATCATACTCACAGCACGTCGGAGGTCCTGGGGGGGAGGGCCCTCTCGGTATCA

-----
121 GGTCTGCGGAACCGGTGAGTACACCGGAATTGCCAGGACGACCGGGTCCTTTCTTGGATC
  CCAGACGCCTTGGCCACTCATGTGGCCTTAACGGTCCTGCTGGCCCAGGAAAGAACCTAG

-----overlap with ag30a-----
#MetProGlyAspLeuGlyValProProGlnAspCysAM
181 AACCCGCTCAATGCCTGGAGATTGGGGCGTGCCCCGCAAGACTGCTAGCCGAGTAGTGT
  TTGGGCGAGTTACGGACCTCTAAACCCGCACGGGGCGTTCTGACGATCGGCTCATACA

-----
                                OP AM GlyAlaCysGluCysProGlyArgSer
                                *
241 TGGGTGCGGAAAGGCCTTGTGGTACTGCCTGATAGGGTGCTTGCGAGTGCCCCGGGAGGT
  ACCCAGCGCTTTCGGGAACACCATGACGGACTATCCCACGAACGCTCACGGGGCCCTCCA

-----
ArgArg
301 CTCGTAGA
  GAGCATCT

```

* = Start of long HCV ORF
 # = Putative small encoded peptides (that may play a translational regulatory role)

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FIG. 15

Translation of DNA 16jh

-----Overlap with 15e-----
1 GlyAlaCysTyrSerIleGluProLeuAspLeuProProIleIleGlnArgLeuHisGly
GGGGCCTGCTACTCCATAGAACCACTGGATCTACCTCCAATCATTCAAAGACTCCATGGC
CCCCGGACGATGAGGTATCTTGGTGACCTAGATGGAGGTTAGTAAGTTTCTGAGGTACCG
61 LeuSerAlaPheSerLeuHisSerTyrSerProGlyGluIleAsnArgValAlaAlaCys
CTCAGCGCATTTTCACTCCACAGTTACTCTCCAGGTGAAATTAATAGGGTGGCCGCATGC
GAGTCGCGTAAAAGTGAGGTGTCAATGAGAGGTCCACTTTAATTATCCCACGGCGTACG
Gly*
G
121 LeuArgLysLeuGlyValProProLeuArgAlaTrpArgHisArgAlaArgSerValArg
CTCAGAAAACCTTGGGGTACCGCCCTTGCGAGCTTGGAGACACCGGGCCCGGAGCGTCCGC
GAGTCTTTTGAACCCCATGGCGGGAACGCTCGAACCTCTGTGGCCCCGGGCTCGCAGGCG
181 AlaArgLeuLeuAlaArgGlyGlyArgAlaAlaIleCysGlyLysTyrLeuPheAsnTrp
GCTAGGCTTCTGGCCAGAGGAGGCAGGGCTGCCATATGTGGCAAGTACCTCTTCAACTGG
CGATCCGAAGACCGGTCTCCTCCGTCCCACGGTATACACCGTTCATGGAGAAGTTGACC
241 AlaValArgThrLysLeuLys
GCAGTAAGAACAAAGCTCAAAC
CGTCATTCTTGTTCGAGTTTG

* = nucleotide heterogeneity

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COMBINED ORF OF DNAs p114a THROUGH 15e

FIG. 16-1

(p114a/CA167b/CA156e/CA84a/CA59a/K9-1/12f/14i/11b/7f/7e/
8h/33c/40b/37b/35/36/81/32/33b/25c/14c/8f/33f/33g/39c/
35f/19g/26g & 15e)

1 ArgSerArgAsnLeuGlyLysValIleAspThrLeuThrCysGlyPheAlaAspLeuMet
AGGTTCGCGCAATTTGGGTAAGGTCATCGATACCCTTACGTGCGGCTTCGCCGACCTCATG
TCCAGCGCGTTAAACCCATTCCAGTAGCTATGGGAATGCACGCCGAAGCGGCTGGAGTAC

61 GlyTyrIleProLeuValGlyAlaProLeuGlyGlyAlaAlaArgAlaLeuAlaHisGly
GGGTACATACCGCTCGTCGGCGCCCTCTTGGAGGCGCTGCCAGGGCCCTGGCGCATGGC
CCCATGTATGGCGAGCAGCCGCGGGGAGAACCTCCGCGACGGTCCCGGGACCGCGTACCG

121 ValArgValLeuGluAspGlyValAsnTyrAlaThrGlyAsnLeuProGlyCysSerPhe
GTCCGGGTTCTGGAAGACGGCGTGAACATATGCAACAGGGAACCTTCCTGGTTGCTCTTTC
CAGGCCCAAGACCTTCTGCCGCACTTGATACGTTGTCCCTTGAAGGACCAACGAGAAAG

181 SerIlePheLeuLeuAlaLeuLeuSerCysLeuThrValProAlaSerAlaTyrGlnVal
TCTATCTTCTTCTGGCCCTGCTCTCTTGCTTGACTGTGCCCGCTTCGGCCTACCAAGTG
AGATAGAAGGAAGACCGGGACGAGAGAACGAACTGACACGGGCGAAGCCGGATGGTTTAC

241 ArgAsnSerThrGlyLeuTyrHisValThrAsnAspCysProAsnSerSerIleValTyr
CGCAACTCCACGGGGCTTTACCACGTCACCAATGATTGCCCTAACTCGAGTATTGTGTAC
GCGTTGAGGTGCCCCGAAATGGTGCAGTGTTACTAACGGGATTGAGCTCATAACACATG

301 GluAlaAlaAspAlaIleLeuHisThrProGlyCysValProCysValArgGluGlyAsn
GAGGCGGCCGATGCCATCCTGCACACTCCGGGGTGCGTCCCTTGCGTTTCGTGAGGGCAAC
CTCCGCCGGCTACGGTAGGACGTGTGAGGCCCCACGCAGGGAACGCAAGCACTCCCGTTG

361 AlaSerArgCysTrpValAlaMetThrProThrValAlaThrArgAspGlyLysLeuPro
GCCTCGAGGTGTTGGGTGGCGATGACCCCTACGGTGGCCACCAGGGATGGCAAACCTCCCC
CGGAGCTCCACAACCCACCGCTACTGGGGATGCCACCGGTGGTCCCTACCGTTTGAGGGG

421 AlaThrGlnLeuArgArgHisIleAspLeuLeuValGlySerAlaThrLeuCysSerAla
GCGACGCAGCTTCGACGTCACATCGATCTGCTTGTGCGGGAGCGCCACCCTCTGTTTCGGCC
CGCTGCGTGAAGCTGCAGTGTAGCTAGACGAACAGCCCTCGCGGTGGGAGACAAGCCGG

481 LeuTyrValGlyAspLeuCysGlySerValPheLeuValGlyGlnLeuPheThrPheSer
CTCTACGTGGGGGACCTATGCGGGTCTGTCTTTCTTGTGCGGCAACTGTTACCTTCTCT
GAGATGACCCCCCTGGATACGCCAGACAGAAAGAACAGCCGGTTGACAAGTGAAGAGA

541 ProArgArgHisTrpThrThrGlnGlyCysAsnCysSerIleTyrProGlyHisIleThr
CCCAGGCGCCACTGGACGACGCAAGGTTGCAATTGCTCTATCTATCCCGGCCATATAACG
GGGTCCGCGGTGACCTGCTGCGTTCCAACGTTAACGAGATAGATAGGGCCGGTATATTGC

601 GlyHisArgMetAlaTrpAspMetMetMetAsnTrpSerProThrThrAlaLeuValMet
GGTCACCGCATGGCATGGGATATGATGATGAACCTGGTCCCTACGACGGCGTGGTAATG
CCAGTGGCGTACCGTACCCTATACTACTTACCAGGGGATGCTGCCGCAACCATTAC

661 AlaGlnLeuLeuArgIleProGlnAlaIleLeuAspMetIleAlaGlyAlaHisTrpGly
GCTCAGCTGCTCCGGATCCACAAGCCATCTTGGACATGATCGCTGGTGCTCACTGGGGA
CGAGTCGACGAGGCCTAGGGTGTTCGGTAGAACCTGTACTAGCGACCACGAGTGACCCCT

721 ValLeuAlaGlyIleAlaTyrPheSerMetValGlyAsnTrpAlaLysValLeuValVal
GTCCTGGCGGGCATAGCGTATTTCTCCATGGTGGGGAACCTGGGCGAAGGTCCTGGTAGTG
CAGGACCGCCCGTATCGCATAAAGAGGTACCACCCCTTGACCCGCTTCCAGGACCATCAC

781 LeuLeuLeuPheAlaGlyValAspAlaGluThrHisValThrGlyGlySerAlaGlyHis
CTGCTGCTATTTGCCGGCGTCGACGCGGAACCCACGTCACCGGGGAAGTGCCGGCCAC
GACGACGATAAACGGCCGAGCTGCGCCTTGGGTGCAGTGGCCCCCTTACGGCCGGTG

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FIG. 16-2

841 ThrValSerGlyPheValSerLeuLeuAlaProGlyAlaLysGlnAsnValGlnLeuIle
ACTGTGTCTGGATTGTGTAGCCTCCTCGCACCAGGCGCCAAGCAGAACGTCCAGCTGATC
TGACACAGACCTAAACAATCGGAGGAGCGTGGTCCGCGGTTCGTCTTGCAGGTCGACTAG

901 AsnThrAsnGlySerTrpHisLeuAsnSerThrAlaLeuAsnCysAsnAspSerLeuAsn
AACACCAACGGCAGTTGGCACCTCAATAGCACGGCCCTGAACTGCAATGATAGCCTCAAC
TTGTGGTTGCCGTCAACCGTGGAGTTATCGTGCCGGGACTTGACGTTACTATCGGAGTTG

961 ThrGlyTrpLeuAlaGlyLeuPheTyrHisHisLysPheAsnSerSerGlyCysProGlu
ACGGCTGGTTGGCAGGGCTTTTCTATCACCACAAGTTCAACTCTTCAGGCTGTCTCTGAG
TGGCCGACCAACCGTCCCGAAAAGATAGTGGTGTTCAGTTGAGAAGTCCGACAGGACTC

1021 ArgLeuAlaSerCysArgProLeuThrAspPheAspGlnGlyTrpGlyProIleSerTyr
AGGCTAGCCAGCTGCCGACCCCTTACCGATTTTGACCAGGGCTGGGGCCCTATCAGTTAT
TCCGATCGGTTCGACGGCTGGGGAATGGCTAAACTGGTCCCGACCCGGGATAGTCAATA

1081 AlaAsnGlySerGlyProAspGlnArgProTyrCysTrpHisTyrProProLysProCys
GCCAACGGAAGCGGCCCGACCGCCCTACTGCTGGCACTACCCCCAAAACCTTGC
CGGTTGCCTTCGCCGGGGCTGGTCCGCGGGATGACGACCGTGATGGGGGGTTTGAACG

1141 GlyIleValProAlaLysSerValCysGlyProValTyrCysPheThrProSerProVal
GGTATTGTGCCCGCAAGAGTGTGTGTGGTCCGGTATATTGCTTCACTCCAGCCCCGTG
CCATAACACGGGCGCTTCTCACACACACCAGGCCATATAACGAAGTGAGGGTCGGGGCAC

1201 ValValGlyThrThrAspArgSerGlyAlaProThrTyrSerTrpGlyGluAsnAspThr
GTGGTGGGAACGACCGAGGTCGGGCGCGCCACCTACAGCTGGGGTGAAAATGATACG
CACCACCCTTGCTGGCTGTCAGCCCGCGGGTGGATGTGACCCCACTTTTACTATGC

1261 AspValPheValLeuAsnAsnThrArgProProLeuGlyAsnTrpPheGlyCysThrTrp
GACGTCTTCGTCTTAACAATACCAGGCCACCGCTGGGCAATTGGTTTCGGTTGTACCTGG
CTGCAGAAGCAGGAATTGTTATGGTCCGGTGGCGACCCGTTAACCAAGCCAACATGGACC

1321 MetAsnSerThrGlyPheThrLysValCysGlyAlaProProCysValIleGlyGlyAla
ATGAACTCAACTGGATTACCAAAGTGTGCGGAGCGCCTCCTTGTTGTCATCGGAGGGGCG
TACTTGAGTTGACCTAAGTGGTTTCACACGCCCTCGCGGAGGAACACAGTAGCCTCCCCGC

1381 GlyAsnAsnThrLeuHisCysProThrAspCysPheArgLysHisProAspAlaThrTyr
GGCAACAACACCCTGCACTGCCCACTGATTGCTTCCGCAAGCATCCGGACGCCACATAC
CCGTTGTTGTGGGACGTGACGGGGTGACTAACGAAGGCGTTTCGTAGGCCTGCGGTGTATG

1441 SerArgCysGlySerGlyProTrpIleThrProArgCysLeuValAspTyrProTyrArg
TCTCGGTGCGGCTCCGGTCCCTGGATCACACCCAGGTGCCTGGTTCGACTACCCGTATAGG
AGAGCCACGCCGAGGCCAGGGACCTAGTGTGGGTCCACGGACCAGCTGATGGGCATATCC

1501 LeuTrpHisTyrProCysThrIleAsnTyrThrIlePheLysIleArgMetTyrValGly
CTTTGGCATTATCCTTGTACCATCAACTACCCATATTTAAATCAGGATGTACGTGGGA
GAAACCGTAATAGGAACATGGTAGTTGATGTGGTATAAATTTTAGTCCTACATGCACCTT

1561 GlyValGluHisArgLeuGluAlaAlaCysAsnTrpThrArgGlyGluArgCysAspLeu
GGGGTGAACACAGGCTGGAAGCTGCCTGCAACTGGACGCGGGGCGAACGTTGCGATCTG
CCCCAGCTTGTTCCGACCTTCGACGGACGTTGACCTGCGCCCCGCTTGCAACGCTAGAC

1621 GluAspArgAspArgSerGluLeuSerProLeuLeuLeuThrThrThrGlnTrpGlnVal
GAAGACAGGGACAGGTCCGAGCTCAGCCCGTTACTGCTGACCACTACACAGTGGCAGGTC
CTTCTGTCCCTGTCCAGGCTCGAGTCGGGCAATGACGACTGGTGTATGTGCACCGTCCAG

1681 LeuProCysSerPheThrThrLeuProAlaLeuSerThrGlyLeuIleHisLeuHisGln
CTCCCGTGTTCCTTCAACCCCTACCAGCCTTGTCCACCGGCCCTCATCCACCTCCACCAG
GAGGGCACAAAGGAAGTGTGGGATGGTCCGAACAGGTGGCCGAGTAGGTGGAGGTGGTC

1741 AsnIleValAspValGlnTyrLeuTyrGlyValGlySerSerIleAlaSerTrpAlaIle
AACATTGTGGACGTGCAGTACTTGTACGGGGTGGGGTCAAGCATCGCGTCTCTGGGCCATT
TTGTAACACCTGCACGTCATGAACATGCCCCACCCAGTTCGTAGCGCAGGACCCGGTAA

1801 LysTrpGluTyrValValLeuLeuPheLeuLeuLeuAlaAspAlaArgValCysSerCys
AAGTGGGAGTACGTCGTTCTCTGTTCTCTGCTTGACAGCGCGCGCTCTGCTCCTGC

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FIG. 16-3

TTCACCCCTCATGCAGCAAGAGGACAAGGAAGACGAACGTCTGCGCGCGCAGACGAGGACG

1861 LeuTrpMetMetLeuLeuIleSerGlnAlaGluAlaAlaLeuGluAsnLeuValIleLeu
TTGTGGATGATGCTACTCATATCCCAAGCGGAGGCGGCTTTGGAGAACCCTCGTAATACTT
AACACCTACTACGATGAGTATAGGGTTCGCCTCCGCCGAAACCTCTTGGAGCATTATGAA

1921 AsnAlaAlaSerLeuAlaGlyThrHisGlyLeuValSerPheLeuValPhePheCysPhe
AATGCAGCATCCCTGGCCGGGACGCACGGTCTTGTATCCTTCCTCGTGTCTTCTGCTTT
TTACGTCGTAGGGACCGGCCCTGCGTGCCAGAACATAGGAAGGAGCACAGAAGACGAAA

1981 AlaTrpTyrLeuLysGlyLysTrpValProGlyAlaValTyrThrPheTyrGlyMetTrp
GCATGGTATTTGAAGGGTAAGTGGGTGCCCGAGCGGTCTACACCTTCTACGGGATGTGG
CGTACCATAAACTTCCCATTACCCACGGGCCTCGCCAGATGTGGAAGATGCCCTACACC

2041 ProLeuLeuLeuLeuLeuAlaLeuProGlnArgAlaTyrAlaLeuAspThrGluVal
CCTCTCCTCCTGCTCCTGTTGGCGTTGCCCGAGCGGCGTACGCGCTGGACACGGAGGTG
GGAGAGGAGGACGAGGACAACCGCAACGGGGTTCGCCGATGCGCGACCTGTGCCTCCAC

2101 AlaAlaSerCysGlyGlyValValLeuValGlyLeuMetAlaLeuThrLeuSerProTyr
GCCGCGTCGTGTGGCGGTGTTGTTCTCGTCGGGTGATGGCGCTGACTCTGTACCATAT
CGGCGCAGCACACCGCCACAACAAGAGCAGCCCACTACCGCGACTGAGACAGTGGTATA

2161 TyrLysArgTyrIleSerTrpCysLeuTrpTrpLeuGlnTyrPheLeuThrArgValGlu
TACAAGCGCTATATCAGCTGGTGCTTGTGGTGGCTTCAGTATTTTCTGACCAGAGTGGAA
ATGTTTCGCGATATAGTCGACCACGAACACCACCGAAGTCATAAAAGACTGGTCTCACCTT

2221 AlaGlnLeuHisValTrpIleProProLeuAsnValArgGlyGlyArgAspAlaValIle
GCGCAACTGCACGTGTGGATTCCCCCCTCAACGTCCGAGGGGGCGCGACGCCGTCATC
CGCGTTGACGTGCACACCTAAGGGGGGAGTTGCAGGCTCCCCCGCGCTGCGGCAGTAG

2281 LeuLeuMetCysAlaValHisProThrLeuValPheAspIleThrLysLeuLeuAla
TTACTCATGTGTGTACACCCGACTCTGGTATTTGACATCACCAAATTGCTGCTGGCC
AATGAGTACACACGACATGTGGGCTGAGACCATAAACTGTAGTGGTTTAACGACGACCGG

2341 ValPheGlyProLeuTrpIleLeuGlnAlaSerLeuLeuLysValProTyrPheValArg
GTCTTCGGACCCCTTTGGATTCTTCAAGCCAGTTTGCTTAAAGTACCTACTTTGTGCGC
CAGAAGCCTGGGGAAACCTAAGAAGTTCGGTCAAACGAATTCATGGGATGAAACACCGC

2401 ValGlnGlyLeuLeuArgPheCysAlaLeuAlaArgLysMetIleGlyGlyHisTyrVal
GTCCAAGGCCTTCTCCGGTCTGCGCGTTAGCGCGGAAGATGATCGGAGGCCATTACGTG
CAGGTTCCGGAAGAGGCCAAGACGCGCAATCGCGCCTTCTACTAGCCTCCGGTAATGCAC

2461 GlnMetValIleIleLysLeuGlyAlaLeuThrGlyThrTyrValTyrAsnHisLeuThr
CAAATGGTCATCATTAAGTTAGGGGCGCTTACTGGCACCTATGTTTATAACCATCTCACT
GTTTACCAGTAGTAATTCAATCCCCGGAATGACCGTGGATACAAATATTGGTAGAGTGA

2521 ProLeuArgAspTrpAlaHisAsnGlyLeuArgAspLeuAlaValAlaValGluProVal
CCTCTTCGGGACTGGGCGCACACGGCTTGCGAGATCTGGCCGTGGCTGTAGAGCCAGTC
GGAGAAGCCCTGACCCGCGTGTGCGGAACGCTCTAGACCGGCACCGACATCTCGGTCAG

2581 ValPheSerGlnMetGluThrLysLeuIleThrTrpGlyAlaAspThrAlaAlaCysGly
GTCTTCTCCCAAATGGAGACCAAGCTCATCAGTGGGGGGCAGATACCGCCGCGTGGGT
CAGAAGAGGGTTTACCTCTGGTTCGAGTAGTGACCCCCCGTCTATGGCGGCGCACGCCA

2641 AspIleIleAsnGlyLeuProValSerAlaArgArgGlyArgGluIleLeuLeuGlyPro
GACATCATCAACGGCTTGCTGTTTCCGCGCGCAGGGGCGGGAGATACTGCTCGGGCCA
CTGTAGTAGTTGCCGAACGGACAAAGGCGGGCGTCCCCGGCCCTCTATGACGAGCCCGGT

2701 AlaAspGlyMetValSerLysGlyTrpArgLeuLeuAlaProIleThrAlaTyrAlaGln
GCCGATGGAATGGTCTCCAAGGGGTGGAGGTTGCTGGCGCCCATCACGGCGTACGCCAG
CGGCTACCTTACCAGAGGTTCCCCACCTCCAACGACCGGGGTAGTCCCGCATGCGGGTC

2761 GlnThrArgGlyLeuLeuGlyCysIleIleThrSerLeuThrGlyArgAspLysAsnGln
CAGACAAGGGGCTCCTAGGGTGCATAATCACCAGCCTAACTGGCCGGGACAAAAACCAA
GTCTGTTCCCGGAGGATCCCACGTATTAGTGGTTCGGATTGACCGGCCCTGTTTTGGTT

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FIG. 16-4

2821 ValGluGlyGluValGlnIleValSerThrAlaAlaGlnThrPheLeuAlaThrCysIle
GTGGAGGGTGAGGTCCAGATTGTGTCAACTGCTGCCCCAACCTTCCTGGCAACGTGCATC
CACCTCCCACTCCAGGTCTAACACAGTTGACGACGGGTTTGAAGGACCGTTGCACGTAG

2881 AsnGlyValCysTrpThrValTyrHisGlyAlaGlyThrArgThrIleAlaSerProLys
AATGGGGTGTGCTGGACTGTCTACCACGGGGCCGGAACGAGGACCATCGCGTCACCCAAG
TTACCCACACGACCTGACAGATGGTGCCCCGGCCTTGCTCCTGGTAGCGCAGTGGGTTC

2941 GlyProValIleGlnMetTyrThrAsnValAspGlnAspLeuValGlyTrpProAlaPro
GGTCCTGTTCATCCAGATGTATACCAATGTAGACCAAGACCTTGTGGGCTGGCCCCGCTCCG
CCAGGACAGTAGGTCTACATATGGTTACATCTGGTTCTGGAACACCCGACCGGGCGAGGC

3001 GlnGlySerArgSerLeuThrProCysThrCysGlySerSerAspLeuTyrLeuValThr
CAAGGTAGCCGCTCATTGACACCCCTGCACTTGCGGCTCCTCGGACCTTTACCTGGTCACG
GTTCCATCGGCGAGTAAGTGTGGGACGTGAACGCCGAGGAGCCTGGAAATGGACAGTGC

3061 ArgHisAlaAspValIleProValArgArgArgGlyAspSerArgGlySerLeuLeuSer
AGGCACGCCGATGTTCATTCCTGCGCCGGCGGGGTGATAGCAGGGGCAGCCTGTGTGCG
TCCGTGCGGCTACAGTAAGGGCACGCGGCCGCCCACTATCGTCCCGTCCGACGACAGC

3121 ProArgProIleSerTyrLeuLysGlySerSerGlyGlyProLeuLeuCysProAlaGly
CCCCGGCCCATTTCTACTTGAAGGCTCCTCGGGGGTCCGCTGTGTGCCCCGCGGGG
GGGGCCGGGTAAAGGATGAACCTTCCGAGGAGCCCCCAGGCGACAACACGGGGCGCCCC

3181 HisAlaValGlyIlePheArgAlaAlaValCysThrArgGlyValAlaLysAlaValAsp
CACGCCGTGGGCATATTTAGGGCCGCGGTGTGCACCCGTGGAGTGGCTAAGCGCGTGGAC
GTGCGGCACCCGTATAAATCCCGCGCCACACGTGGGCACCTCACCGATTCCGCCACCTG

3241 PheIleProValGluAsnLeuGluThrThrMetArgSerProValPheThrAspAsnSer
TTTATCCCTGTGGAGAACCTAGAGACAACCATGAGGTCCCCGGTGTTCACGGATAACTCC
AAATAGGGACACCTCTTGGATCTCTGTTGTTACTCCAGGGGCCACAAGTGCCTATTGAGG

3301 SerProProValValProGlnSerPheGlnValAlaHisLeuHisAlaProThrGlySer
TCTCCACCACTAGTGCCCCAGAGCTTCAGGTGGCTCACCTCCATGCTCCACAGGCAGC
AGAGGTGGTCATCACGGGTCTCGAAGGTCCACCGAGTGGAGGTACGAGGGTGTCCGTCG

3361 GlyLysSerThrLysValProAlaAlaTyrAlaAlaGlnGlyTyrLysValLeuValLeu
GGCAAAAGCACCAAGGTCCCGGCTGCATATGCAGCTCAGGGCTATAAGGTGCTAGTACTC
CCGTTTTCTGTGTTCCAGGGCCGACGTATACGTGAGTCCCGATATTCCACGATCATGAG

3421 AsnProSerValAlaAlaThrLeuGlyPheGlyAlaTyrMetSerLysAlaHisGlyIle
AACCCCTCTGTTGCTGCAACACTGGGCTTTGGTGCTTACATGTCCAAGGCTCATGGGATC
TTGGGGAGACAACGACGTTGTGACCCGAAACCAGGAATGTACAGGTTCCGAGTACCCTAG

3481 AspProAsnIleArgThrGlyValArgThrIleThrThrGlySerProIleThrTyrSer
GATCCTAACATCAGGACCGGGGTGAGAACAATTACCACTGGCAGCCCCATCACGTACTCC
CTAGGATTGTAGTCTTGCCCCACTCTTGTTAATGGTGACCGTGGGGTAGTGCATGAGG

3541 ThrTyrGlyLysPheLeuAlaAspGlyGlyCysSerGlyGlyAlaTyrAspIleIleIle
ACCTACGGCAAGTTCCTTGCCGACGGCGGGTGCTCGGGGGGCGCTTATGACATAATAATT
TGGATGCCGTCAAGGAACGGCTGCCGCCACGAGCCCCCGGAATACTGTATTATTAA

3601 CysAspGluCysHisSerThrAspAlaThrSerIleLeuGlyIleGlyThrValLeuAsp
TGTGACGAGTGCCACTCCACGGATGCCACATCCATCTTGGGCATCGGCACTGTCCTTGAC
ACACTGCTCACGGTGAGGTGCCTACGGTGTAGGTAGAACCCGTAGCCGTGACAGGAAGT

3661 GlnAlaGluThrAlaGlyAlaArgLeuValValLeuAlaThrAlaThrProProGlySer
CAAGCAGAGACTGCGGGGGCGAGACTGGTTGTGCTCGCCACCGCCACCCCTCCGGGCTCC
GTTGCTCTCTGACGCCCCGCTCTGACCAACACGAGCGGTGGCGGTGGGGAGGCCGAGG

3721 ValThrValProHisProAsnIleGluGluValAlaLeuSerThrThrGlyGluIlePro
GTCACTGTGCCCCATCCCAACATCGAGGAGGTGCTCTGTCCACCACCGGAGAGATCCCT
CAGTGACACGGGGTAGGGTTGTAGCTCCTCCAACGAGACAGGTGGTGGCCTCTCTAGGGA

3781 PheTyrGlyLysAlaIleProLeuGluValIleLysGlyGlyArgHisLeuIlePheCys
TTTTACGGCAAGGCTATCCCCCTCGAAGTAATCAAGGGGGGAGACATCTCATCTCTGT

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FIG. 16-5

AAAATGCCGTTCCGATAGGGGGAGCTTCATTAGTTCCCCCCTCTGTAGAGTAGAAGACA

3841 HisSerLysLysLysCysAspGluLeuAlaAlaLysLeuValAlaLeuGlyIleAsnAla
CATTCAAAGAAGAAGTGCACGAACTCGCCGCAAAGCTGGTCGCATTGGGCATCAATGCC
GTAAGTTTCTTCTTCACGCTGCTTGAGCGGCGTTTCGACCAGCGTAACCCGTAGTTACGG

3901 ValAlaTyrTyrArgGlyLeuAspValSerValIleProThrSerGlyAspValValVal
GTGGCCTACTACCGCGGTCTTGACGTGTCCGTCATCCCGACCAGCGCGCATGTTGTCTGTC
CACCGGATGATGGCGCCAGAACTGCACAGGCAGTAGGGCTGGTCGCCGCTACAACAGCAG

3961 ValAlaThrAspAlaLeuMetThrGlyTyrThrGlyAspPheAspSerValIleAspCys
GTGGCAACCGATGCCCTCATGACCGGCTATACCGGCGACTTCGACTCGGTGATAGACTGC
CACCGTTGGCTACGGGAGTACTGGCCGATATGGCCGCTGAAGCTGAGCCACTATCTGACG

4021 AsnThrCysValThrGlnThrValAspPheSerLeuAspProThrPheThrIleGluThr
AATACGTGTGTACCCAGACAGTCGATTTTCAGCCTTGACCCTACCTTACCATTGAGACA
TTATGCACACAGTGGGTCTGTCTAGCTAAAGTCGGAACCTGGGATGGAAGTGGAAGTCTGT

4081 IleThrLeuProGlnAspAlaValSerArgThrGlnArgArgGlyArgThrGlyArgGly
ATCACGCTCCCCAGGATGCTGTCTCCCGCACTCAACGTCGGGGCAGGACTGGCAGGGGG
TAGTGCAGGGGGTCTTACGACAGAGGGCGTGAGTTGCAGCCCCGTCCTGACCGTCCCCC

4141 LysProGlyIleTyrArgPheValAlaProGlyGluArgProSerGlyMetPheAspSer
AAGCCAGGCATCTACAGATTGTGGCACCGGGGAGCGCCCCCTCCGGCATGTTTCCACTCG
TTCGGTCCGTAGATGTCTAAACACCGTGGCCCCCTCGCGGGGAGGCCGTACAAGCTGAGC

4201 SerValLeuCysGluCysTyrAspAlaGlyCysAlaTrpTyrGluLeuThrProAlaGlu
TCCGTCTCTGTGAGTGCTATGACGCAGGCTGTGCTTGGTATGAGCTCACGCCGCCGAG
AGGCAGGAGACACTCACGATACTGCGTCCGACACGAACCATACTCGAGTGCGGGCGGCTC

4261 ThrThrValArgLeuArgAlaTyrMetAsnThrProGlyLeuProValCysGlnAspHis
ACTACAGTTAGGCTACGAGCGTACATGAACACCCCGGGGCTTCCCGTGTGCCAGGACCAT
TGATGTCAATCCGATGCTCGCATGTACTTGTGGGGCCCCGAAGGGCACACGGTCTTGTA

4321 LeuGluPheTrpGluGlyValPheThrGlyLeuThrHisIleAspAlaHisPheLeuSer
CTTGAATTTTGGGAGGGCGTCTTTACAGGCCTCACTCATATAGATGCCACTTTCTATCC
GAACTTAAACCCCTCCCGCAGAAATGTCCGGAGTGAGTATATCTACGGGTGAAAGATAGG

4381 GlnThrLysGlnSerGlyGluAsnLeuProTyrLeuValAlaTyrGlnAlaThrValCys
CAGACAAAGCAGAGTGGGGAGAACCTTCTTACCTGGTAGCGTACCAAGCCACCGTGTGC
GTCTGTTTTCTCTCACCCCTCTTGAAGGAATGGACCATCGCATGGTTCCGTGGCACACC

4441 AlaArgAlaGlnAlaProProProSerTrpAspGlnMetTrpLysCysLeuIleArgLeu
GCTAGGGCTCAAGCCCCTCCCCATCGTGGGACCAGATGTGGAAGTGTGATTGCGCTC
CGATCCCGAGTTCGGGGAGGGGTAGCACCTGGTCTACACCTTCAAACTAAGCGGAG

4501 LysProThrLeuHisGlyProThrProLeuLeuTyrArgLeuGlyAlaValGlnAsnGlu
AAGCCCACCCTCCATGGGCCAACCCCTGCTATACAGACTGGGCGCTGTTTCAAGATGAA
TTCGGGTGGGAGGTACCCGTTGTGGGGACGATATGTCTGACCCGCGACAAGTCTTACTT

4561 IleThrLeuThrHisProValThrLysTyrIleMetThrCysMetSerAlaAspLeuGlu
ATCACCTGACGCACCCAGTCACCAAATACATCATGACATGATGTCGGCCGACCTGGAG
TAGTGGGACTGCGTGGGTCACTGGTTTATGTAGTACTGTACGTACAGCCGGCTGGACCTC

4621 ValValThrSerThrTrpValLeuValGlyGlyValLeuAlaAlaLeuAlaAlaTyrCys
GTCGTACAGACACCTGGGTGCTCGTTGGCGGCGTCTGGCTGCTTTGGCCGCGTATTGC
CAGCAGTGCTCGTGGACCCACGAGCAACCGCCGAGGACCGACGAAACGGGCGCATAACG

4681 LeuSerThrGlyCysValValIleValGlyArgValValLeuSerGlyLysProAlaIle
CTGTCAACAGGCTGCGTGGTTCATAGTGGGCAGGGTCGTCTGTCCGGGAAGCCGGCAATC
GACAGTTGTCCGACGCACCAAGTATCACCCGTCCAGCAGAACAGGCCCTTCGGCCGTTAG

4741 IleProAspArgGluValLeuTyrArgGluPheAspGluMetGluGluCysSerGlnHis
ATACCTGACAGGGAAGTCTCTACCGAGAGTTTCGATGAGATGGAAGAGTCTCTCAGCAC
TATGGACTGTCCCTTACAGGAGATGGCTCTCAAGCTACTCTACCTTCTCAGAGAGTCTGT

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FIG. 16-6

4801 LeuProTyrIleGluGlnGlyMetMetLeuAlaGluGlnPheLysGlnLysAlaLeuGly
TTACCGTACATCGAGCAAGGGATGATGCTCGCCGAGCAGTTCAAGCAGAAGGCCCTCGGC
AATGGCATGTAGCTCGTTCCTACTACGAGCGGCTCGTCAAGTTCGTCTTCCGGGAGCCG

4861 LeuLeuGlnThrAlaSerArgGlnAlaGluValIleAlaProAlaValGlnThrAsnTrp
CTCCTGCAGACCGCGTCCCGTCAGGCAGAGGTTATCGCCCCTGCTGTCCAGACCAACTGG
GAGGACGTCTGGCGCAGGGCAGTCCGTCTCCAATAGCGGGACGACAGGTCTGGTTGACC

4921 GlnLysLeuGluThrPheTrpAlaLysHisMetTrpAsnPheIleSerGlyIleGlnTyr
CAAAAACCTCGAGACCTTCTGGGCGAAGCATATGTGGAACCTCATCAGTGGGATACAATAC
GTTTTTGAGCTCTGGAAGACCCGCTTCGTATACACCTTGAAGTAGTCACCCATGTTATG

4981 LeuAlaGlyLeuSerThrLeuProGlyAsnProAlaIleAlaSerLeuMetAlaPheThr
TTGGCGGGCTTGTCAACGCTGCCTGGTAACCCCGCCATTGCTTCATTGATGGCTTTTACA
AACCGCCCGAACAGTTGCGACGGACCATTGGGGCGGTAAACGAACTAACCGAAAAATGT

5041 AlaAlaValThrSerProLeuThrThrSerGlnThrLeuLeuPheAsnIleLeuGlyGly
GCTGCTGTCAACGACCCACTAACCCTAGCCAAACCCCTCCTCTCAACATATTGGGGGGG
CGACGACAGTGGTCGGGTGATTGGTGATCGGTTTGGGAGGAGAAGTTGTATAACCCCCC

5101 TrpValAlaAlaGlnLeuAlaAlaProGlyAlaAlaThrAlaPheValGlyAlaGlyLeu
TGGGTGGCTGCCAGCTCGCCGCCCCCGGTGCCGCTACTGCCTTTGTGGGCGCTGGCTTA
ACCCACCGACGGGTGAGCGGGCGGGGCCACGGCGATGACGAAACACCCGCGACCGAAT

5161 AlaGlyAlaAlaIleGlySerValGlyLeuGlyLysValLeuIleAspIleLeuAlaGly
GCTGGCGCGGCCATCGGCAGTGTGGACTGGGGAAGGTCCTCATAGACATCCTTGACGGG
CGACCGCGGGTAGCCGTCACAACCTGACCCCTTCCAGGAGTATCTGTAGGAACGTCCC

5221 TyrGlyAlaGlyValAlaGlyAlaLeuValAlaPheLysIleMetSerGlyGluValPro
TATGGCGCGGGCGTGGCGGGAGCTCTTGTGGCATTCAAGATCATGAGCGGTGAGGTCCCC
ATACCGCGCCCGCACCGCCCTCGAGAACACCGTAAGTTCTAGTACTCGCCACTCCAGGGG

5281 SerThrGluAspLeuValAsnLeuLeuProAlaIleLeuSerProGlyAlaLeuValVal
TCCACGGAGGACCTGGTCAATCTACTGCCCGCCATCCTCTCGCCCGGAGCCCTCGTAGTC
AGGTGCCTCCTGGACAGTTAGATGACGGGCGGTAGGAGAGCGGGCCTCGGGAGCATCAG

5341 GlyValValCysAlaAlaIleLeuArgArgHisValGlyProGlyGluGlyAlaValGln
GGCGTGGTCTGTGCAGCAATACTGCGCCGGCACGTTGGCCCCGGGCGAGGGGGCAGTGCAG
CCGCACCAGACACGTGCTTATGACGGCGCGGTGCAACCGGGCCCGCTCCCCCGTCACGTC

5401 TrpMetAsnArgLeuIleAlaPheAlaSerArgGlyAsnHisValSerProThrHisTyr
TGGATGAACCGGCTGATAGCCTTCGCCTCCCGGGGAACCATGTTTCCCCCAGCCTAC
ACCTACTTGGCCGACTATCGGAAGCGGAGGGCCCCCTTGGTACAAAGGGGGTGCGTGATG

5461 ValProGluSerAspAlaAlaAlaArgValThrAlaIleLeuSerSerLeuThrValThr
GTGCCGGAGAGCGATGCAGCTGCCCGCTCACTGCCATACTCAGCAGCCTCACTGTAACC
CACGGCCTCTCGCTACGTGACGGGCGCAGTGACGGTATGAGTCGTGCGAGTGACATTGG

5521 GlnLeuLeuArgArgLeuHisGlnTrpIleSerSerGluCysThrThrProCysSerGly
CAGCTCCTGAGGCGACTGCACCACTGGATAAGCTCGGAGTGTAACCTCCATGCTCCGGT
GTCGAGGACTCCGCTGACGTGGTCACCTATTTCGAGCCTCACATGGTGAGGTACGAGGCCA

5581 SerTrpLeuArgAspIleTrpAspTrpIleCysGluValLeuSerAspPheLysThrTrp
TCCTGGCTAAGGGACATCTGGGACTGGATATGCGAGGTGTGAGCGACTTTAAGACCTGG
AGGACCGATTCCCTGTAGACCCTGACCTATACGCTCCACAACCTCGCTGAAATTCTGGACC

5641 LeuLysAlaLysLeuMetProGlnLeuProGlyIleProPheValSerCysGlnArgGly
CTAAAAGCTAAGCTCATGCCACAGCTGCCTGGGATCCCCCTTGTGTCCTGCCAGCGCGGG
GATTTTCGATTTCGAGTACGGTGTGACGGACCCCTAGGGGAAACACAGGACGGTCGCGCCC

5701 TyrLysGlyValTrpArgValAspGlyIleMetHisThrArgCysHisCysGlyAlaGlu
TATAAGGGGGTCTGGCGAGTGGACGGCATCATGCACACTCGCTGCCACTGTGGAGCTGAG
ATATTCCCCCAGACCGCTCACCTGCCGTAGTACGTGTGAGCGACGGTGACACCTCGACTC

5761 IleThrGlyHisValLysAsnGlyThrMetArgIleValGlyProArgThrCysArgAsn
ATCACTGGACATGTCAAAAACGGGACGATGAGGATCGTCGGTCCTAGGACCTGCAGGAAC

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FIG. 16-7

TAGTGACCTGTACAGTTTTTGCCTGCTACTCCTAGCAGCCAGGATCCTGGACGTCCTTG

5821 MetTrpSerGlyThrPheProIleAsnAlaTyrThrThrGlyProCysThrProLeuPro
ATGTGGAGTGGGACCTTCCCCATTAATGCCTACACCACGGGCCCCGTACCCCCCTTCCT
TACACCTCACCTTGAAGGGTAATTACGGATGTGGTGGCCGGGGACATGGGGGGAAGGA

5881 AlaProAsnTyrThrPheAlaLeuTrpArgValSerAlaGluGluTyrValGluIleArg
GCGCCGAACCTACAGTTTCGCGCTATGGAGGGTGTCTGCAGAGGAATATGTGGAGATAAGG
CGCGGCTTGATGTGCAAGCGGATACCTCCACAGACGTCTCCTTATACACCTCTATTCC

5941 GlnValGlyAspPheHisTyrValThrGlyMetThrThrAspAsnLeuLysCysProCys
CAGGTGGGGGACTTCCACTACGTGACGGGTATGACTACTGACAATCTCAAATGCCCCGTGC
GTCCACCCCTGAAGGTGATGCACTGCCCATACTGATGACTGTTAGAGTTTACGGGCACG

6001 GlnValProSerProGluPhePheThrGluLeuAspGlyValArgLeuHisArgPheAla
CAGGTCCCATCGCCGAATTTTTCACAGAATTGGACGGGGTGCGCCTACATAGGTTTGGCG
GTCCAGGGTAGCGGGCTTAAAAAGTGTCTTAACCTGCCCCACGCGGATGTATCCAAACGC

6061 ProProCysLysProLeuLeuArgGluGluValSerPheArgValGlyLeuHisGluTyr
CCCCCTGCAAGCCCTTGCTGCGGGAGGAGGTATCATTAGAGTAGGACTCCACGAATAC
GGGGGACGTTTCGGGAACGACGCCCTCCTCCATAGTAAGTCTCATCTGAGGTGCTTATG

6121 ProValGlySerGlnLeuProCysGluProGluProAspValAlaValLeuThrSerMet
CCGGTAGGGTCGCAATTACCTTGCGAGCCCGAACCGGACGTGGCCGTGTTGACGTCCATG
GGCCATCCCAGCGTTAATGGAACGCTCGGGCTTGGCCTGCACCGGCACAACCTGCAGGTAC

6181 LeuThrAspProSerHisIleThrAlaGluAlaAlaGlyArgArgLeuAlaArgGlySer
CTCACTGATCCCTCCCATATAACAGCAGAGGCGGGCCGCGGCGAAGGTTGGCGAGGGGATCA
GAGTGACTAGGGAGGGTATATTGTCGTCTCCGCGGGCCGCTTCCAACCGCTCCCTAGT

6241 ProProSerValAlaSerSerSerAlaSerGlnLeuSerAlaProSerLeuLysAlaThr
CCCCCTCTGTGGCCAGCTCCTCGGCTAGCCAGCTATCCGCTCCATCTCTCAAGGCAACT
GGGGGAGACACCGGTTCGAGGAGCCGATCGGTTCGATAGGCGAGGTAGAGAGTTCGGTTGA

6301 CysThrAlaAsnHisAspSerProAspAlaGluLeuIleGluAlaAsnLeuLeuTrpArg
TGCACCGCTAACCATGACTCCCTGATGCTGAGCTCATAGAGGCCAACCTCCTATGGAGG
ACGTGGCGATTGGTACTGAGGGGACTACGACTCGAGTATCTCCGGTTGGAGGATACCTCC

6361 GlnGluMetGlyGlyAsnIleThrArgValGluSerGluAsnLysValValIleLeuAsp
CAGGAGATGGGCGGCAACATCACCAGGGTTGAGTCAGAAAACAAAGTGGTGATTCTGGAC
GTCTCTACCCGCCGTTGTAGTGGTCCCAACTCAGTCTTTTGTTCACCACTAAGACCTG

6421 SerPheAspProLeuValAlaGluGluAspGluArgGluIleSerValProAlaGluIle
TCCTTCGATCCGCTTGTGGCGGAGGAGGACGAGCGGGAGATCTCCGTACCCGCAGAAATC
AGGAAGCTAGGCGAACACCGCCTCCTCTGCTCGCCCTCTAGAGGCATGGGCGTCTTTAG

6481 LeuArgLysSerArgArgPheAlaGlnAlaLeuProValTrpAlaArgProAspTyrAsn
CTGCGGAAGTCTCGGAGATTGCGCCAGGCCCTGCCCGTTTGGGCGCGGCCGACTATAAC
GACGCCTTCAGAGCCTCTAAGCGGGTCCGGGACGGGCAAACCCGCGCCGGCCTGATATTG

6541 ProProLeuValGluThrTrpLysLysProAspTyrGluProProValValHisGlyCys
CCCCCGCTAGTGGAGACGTGGAAAAAGCCCGACTACGAACCACCTGTGGTCCATGGCTGT
GGGGGCGATCACCTCTGCACCTTTTTTCGGGCTGATGCTTGGTGGACACCAGGTACCGACA

6601 ProLeuProProProLysSerProProValProProProArgLysLysArgThrValVal
CCGCTTCCACCTCCAAAGTCCCTCCTGTGCCTCCGCCTCGGAAGAAGCGGACGGTGGTC
GGCGAAGGTGGAGGTTTCAGGGGAGGACACGGAGGCGGAGCCTTCTTCGCTGCCACCAG

6661 LeuThrGluSerThrLeuSerThrAlaLeuAlaGluLeuAlaThrArgSerPheGlySer
CTCACTGAATCAACCCTATCTACTGCCTTGGCCGAGCTCGCCACCAGAAGCTTTGGCAGC
GAGTGACTTAGTTGGGATAGATGACGGAACCGGCTCGAGCGGTGGTCTTCGAAACCGTCG

6721 SerSerThrSerGlyIleThrGlyAspAsnThrThrThrSerSerGluProAlaProSer
TCCTCAACTTCCGGCATTACGGGCGACAATACGACAACATCCTCTGAGCCCGCCCTTCT
AGGAGTTGAAGGCGTAATGCCCGCTGTTATGCTGTTGTAGGAGACTCGGGCGGGGAAGA

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FIG. 16-8

6781 GlyCysProProAspSerAspAlaGluSerTyrSerSerMetProProLeuGluGlyGlu
GGTGCCCCCGACTCCGACGCTGAGTCCTATTCTCCATGCCCCCCTGGAGGGGGAG
CCGACGGGGGGGCTGAGGCTGCGACTCAGGATAAGGAGGTACGGGGGGGACCTCCCCCTC

6841 ProGlyAspProAspLeuSerAspGlySerTrpSerThrValSerSerGluAlaAsnAla
CCTGGGGATCCGGATCTTAGCGACGGGTTCATGGTCAACGGTCAGTAGTGAGGCCAACGCG
GGACCCCTAGGCCTAGAATCGCTGCCCAGTACCAGTTGCCAGTCATCACTCCGGTTGCGC

6901 GluAspValValCysCysSerMetSerTyrSerTrpThrGlyAlaLeuValThrProCys
GAGGATGTCGTGTGCTGCTCAATGTCTTACTCTTGGACAGGCGCACTCGTCACCCCGTGC
CTCCTACAGCACACGACGAGTTACAGAATGAGAACCTGTCCGCGTGAGCAGTGGGGCAGC

6961 AlaAlaGluGluGlnLysLeuProIleAsnAlaLeuSerAsnSerLeuLeuArgHisHis
GCCGCGGAAGAAGAACTGCCCATCAATGCACTAAGCAACTCGTTGCTACGTCACCAC
CGGCGCCTTCTTGTCTTTGACGGGTAGTTACGTGATTCTGTTGAGCAACGATGCAGTGGTG

7021 AsnLeuValTyrSerThrThrSerArgSerAlaCysGlnArgGlnLysLysValThrPhe
AATTTGGTGTATTCCACCACCTCAGCGAGTGCTTGCCAAAGGCAGAAAGTCACATTT
TTAAACCACATAAGGTGGTGGAGTGCCTACGAACGGTTTCCGTCTTCTTTCAGTGTTAA

7081 AspArgLeuGlnValLeuAspSerHisTyrGlnAspValLeuLysGluValLysAlaAla
GACAGACTGCAAGTTCTGGACAGCCATTACCAGGACGTACTCAAGGAGGTTAAAGCAGCG
CTGTCTGACGTTCAAGACCTGTCCGGTAATGGTCTGCATGAGTTCCTCCAATTTCTGCGC

7141 AlaSerLysValLysAlaAsnLeuLeuSerValGluGluAlaCysSerLeuThrProPro
GCGTCAAAAGTGAAGGCTAACTTGCTATCCGTAGAGGAAGCTTGCAGCCTGACGCCCCCA
CGCAGTTTTCACCTCCGATTGAACGATAGGCATCTCCTTCGAACGTCCGACTGCGGGGGT

7201 HisSerAlaLysSerLysPheGlyTyrGlyAlaLysAspValArgCysHisAlaArgLys
CACTCAGCCAAATCCAAGTTTGGTTATGGGGCAAAGACGTCCGTTGCCATGCCAGAAAG
GTGAGTCGGTTTAGGTTCAAACCAATACCCCGTTTTCTGCAGGCAACGGTACGGTCTTTC

7261 AlaValThrHisIleAsnSerValTrpLysAspLeuLeuGluAspAsnValThrProIle
GCCGTAACCCACATCAACTCCGTGTGGAAAGACCTTCTGGAAGACAATGTAAACCAATA
CGGCATTGGGTGTAGTTGAGGCACACCTTTCTGGAAGACCTTCTGTTACATTGTGGTTAT

7321 AspThrThrIleMetAlaLysAsnGluValPheCysValGlnProGluLysGlyGlyArg
GACACTACCATCATGGCTAAGAACGAGGTTTTCTGCGTTTCAGCCTGAGAAGGGGGTTCGT
CTGTGATGGTAGTACCGATTCTTGCTCCAAAAGACGCAAGTCGGACTCTTCCCCCAGCA

7381 LysProAlaArgLeuIleValPheProAspLeuGlyValArgValCysGluLysMetAla
AAGCCAGCTCGTCTCATCGTGTTCCTCGATCTGGGCGTGCGCGTGTGCGAAAAGATGGCT
TTCGGTCGAGCAGAGTAGCACAAGGGGCTAGACCCGCACGCGCACAGCTTTTCTACCGA

7441 LeuTyrAspValValThrLysLeuProLeuAlaValMetGlySerSerTyrGlyPheGln
TTGTACGACGTGGTTACAAAGCTCCCCTTGGCCGTGATGGGAAGCTCCTACGGATTCCAA
AACATGTGTCACCAATGTTTCGAGGGGAACCGGCCTACCTTCGAGGATGCCTAAGGTT

7501 TyrSerProGlyGlnArgValGluPheLeuValGlnAlaTrpLysSerLysLysThrPro
TACTCACCAGGACAGCGGGTTGAATTCCTCGTGCAAGCTGGAAGTCCAAGAAAACCCCA
ATGAGTGGTCTGTGCGCCAACTTAAGGAGCACGTTCCGACCTTCAGGTTCTTTTGGGGT

7561 MetGlyPheSerTyrAspThrArgCysPheAspSerThrValThrGluSerAspIleArg
ATGGGGTTCTCGTATGATACCCGCTGCTTTGACTCCACAGTCACTGAGAGCGACATCCGT
TACCCCAAGAGCATACTATGGGCGACGAACTGAGGTGTCAGTACTCTCGCTGTAGGCA

7621 ThrGluGluAlaIleTyrGlnCysCysAspLeuAspProGlnAlaArgValAlaIleLys
ACGGAGGAGGCAATCTACCAATGTTGTGACCTCGACCCCCAAGCCCGGTGGCCATCAAG
TGCTCTCTCCGTTAGATGGTTACAACACTGGAGCTGGGGGTTCCGGGCGCACCGGTAGTTC

7681 SerLeuThrGluArgLeuTyrValGlyGlyProLeuThrAsnSerArgGlyGluAsnCys
TCCCTCACCAGAGGCTTTATGTTGGGGGCCCTTACCAATTCAAGGGGGGAGAACTGC
AGGGAGTGGCTCTCCGAAATACAACCCCGGGAGAAATGGTTAAGTTCCCCCTCTTGACG

7741 GlyTyrArgArgCysArgAlaSerGlyValLeuThrThrSerCysGlyAsnThrLeuThr
GGCTATCGCAGGTGCCGCGGAGCGGCTACTGACAACCTAGCTGTGGTAACACCCCTCACT

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FIG. 16-9

CCGATAGCGTCCACGGCGCGCTCGCCGCATGACTGTTGATCGACACCATTGTGGGAGTGA

7801 CysTyrIleLysAlaArgAlaAlaCysArgAlaAlaGlyLeuGlnAspCysThrMetLeu
TGCTACATCAAGGCCCGGGCAGCCTGTGAGCCGCAGGGCTCCAGGACTGCACCATGTCTC
ACGATGTAGTTCCGGGCCCCGTGCGACAGCTCGGCGTCCCGAGGTCCTGACGTGGTACGAG

7861 ValCysGlyAspAspLeuValValIleCysGluSerAlaGlyValGlnGluAspAlaAla
GTGTGTGGCGACGACTTAGTCGTTATCTGTGAAAGCGCGGGGGTCCAGGAGGACGCGGCG
CACACACCGCTGCTGAATCAGCAATAGACACTTTCGCGCCCCCAGGTCTCTCTGCGCCCG

7921 SerLeuArgAlaPheThrGluAlaMetThrArgTyrSerAlaProProGlyAspProPro
AGCCTGAGAGCCTTCACGGAGGCTATGACCAGGTACTCCGCCCCCCTGGGGACCCCCCA
TCGGACTCTCGGAAGTGCCTCCGATACTGGTCCATGAGGCGGGGGGACCCCTGGGGGGT

7981 GlnProGluTyrAspLeuGluLeuIleThrSerCysSerSerAsnValSerValAlaHis
CAACCAGAATACGACTTGGAGCTCATAACATCATGCTCCTCCAACGTGTCTAGTCGCCCCAC
GTTGGTCTTATGCTGAACCTCGAGTATTGTAGTACGAGGAGGTTGCACAGTCAGCGGGTG

8041 AspGlyAlaGlyLysArgValTyrTyrLeuThrArgAspProThrThrProLeuAlaArg
GACGGCGCTGGAAAGAGGGTCTACTACCTCACCCGTGACCCTACAACCCCCCTCGCGAGA
CTGCCGCGACCTTCTCCAGATGATGGAGTGGGCACTGGGATGTTGGGGGGAGCGCTCT

8101 AlaAlaTrpGluThrAlaArgHisThrProValAsnSerTrpLeuGlyAsnIleIleMet
GCTGCGTGGGAGACAGCAAGACACACTCCAGTCAATTCCTGGCTAGGCAACATAATCATG
CGACGCACCCTCTGTCGTTCTGTGTGAGGTCAGTTAAGGACCGATCCGTTGTATTAGTAC

8161 PheAlaProThrLeuTrpAlaArgMetIleLeuMetThrHisPhePheSerValLeuIle
TTTGGCCCCACACTGTGGGCGAGGATGATACTGATGACCCATTTCTTTAGCGTCTCTTATA
AAACGGGGGTGTGACACCCGCTCCTACTATGACTACTGGGTAAAGAAATCGCAGGAATAT

8221 AlaArgAspGlnLeuGluGlnAlaLeuAspCysGluIleTyrGlyAlaCysTyrSerIle
GCCAGGGACCAGCTTGAACAGGCCCTCGATTGCGAGATCTACGGGGCCTGCTACTCCATA
CGTCCCTGGTCGAACTTGTCCGGGAGCTAACGCTCTAGATGCCCCGGACGATGAGGTAT

8281 GluProLeuAspLeuProProIleIleGlnArgLeu
GAACCACTTGATCTACCTCCAATCATTCAAAGACTC
CTTGGTGAAC TAGATGGAGGTTAGTAAGTTTCTGAG

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FIG. 17-1

- 319 CACTCCACCATGAATCACTCCCCCTGTGAGGAACCTACTGTCTTCACGCAGAAAGCGTCTAG
GTGAGGTGGTACTTAGTGAGGGGACACTCCTTGATGACAGAAGTGCCTCTTTTCGCAGATC
- 259 CCATGGCGTTAGTATGAGTGTGCGTCAGCCTCCAGGACCCCCCTCCCGGGAGAGCCATA
GGTACCGCAATCATACTCACAGCAGCTCGGAGGTCTGGGGGGGAGGGCCCTCTCGGTAT
- 199 GTGGTCTGCGGAACCGGTGAGTACACCGGAATTGCCAGGACGACCGGGTCCCTTTCTTGGA
CACCAGACGCCTTGCCACTCATGTGGCCTTAACGGTCTGCTGGCCCAGGAAAGAACCT
- 139 TCAACCCGCTCAATGCCTGGAGATTGGGGCGTCCCCCGCAAGACTGCTAGCCGAGTAGT
AGTTGGGCGAGTTACGGACCTCTAAACCCGCACGGGGCGTTCTGACGATCGGCTCATCA
- 79 GTTGGGTGCGGAAAGGCCTTGTGGTACTGCCTGATAGGGTGCTTGCGAGTGCCCCGGGAG
CAACCCAGCGCTTTCGGAACACCATGACGGACTATCCACGAACGCTCACGGGGCCCTC
- 19 GTCTCGTAGACCGTGCACC
CAGAGCATCTGGCACGTGG
- Arg Thr
- 1 MetSerThrAsnProLysProGlnLysLysAsnLysArgAsnThrAsnArgArgProGln
ATGAGCACGAATCCTAAACCTCAAAAAAACAACGTAACACCAACCGTCGCCCACAG
TACTCGTGCTTAGGATTTGGAGTTTTTTTTTTGTTTGCATTGTGGTTGGCAGCGGGTGTC
- 61 AspValLysPheProGlyGlyGlyGlnIleValGlyGlyValTyrLeuLeuProArgArg
GACGTCAAGTTCCCGGGTGGCGGTGAGATCGTTGGTGGAGTTTACTTGTGTGCCGCGCAGG
CTGCAGTTCAAGGGCCACCGCCAGTCTAGCAACCACCTCAAATGAACAACGGCGCGTCC
- 121 GlyProArgLeuGlyValArgAlaThrArgLysThrSerGluArgSerGlnProArgGly
GGCCCTAGATTGGGTGTGCGCGCGACGAGAAAGACTTCCGAGCGGTGCGAACCTCGAGGT
CCGGGATCTAACCACACGCGCGTGTCTTTCTGAAGGCTCGCCAGCGTTGGAGCTCCA
- 181 ArgArgGlnProIleProLysAlaArgArgProGluGlyArgThrTrpAlaGlnProGly
AGACGTGAGCTATCCCCAAGGCTCGTCGGCCCCGAGGGCAGGACCTGGGCTCAGCCCCGGG
TCTGCAGTCGGATAGGGTTCCGAGCAGCGGGCTCCCGTCTGGACCCGAGTCGGGGCC
- 241 TyrProTrpProLeuTyrGlyAsnGluGlyCysGlyTrpAlaGlyTrpLeuLeuSerPro
TACCCTTGGCCCCCTCTATGGCAATGAGGGCTGCGGGTGGGCGGGATGGCTCCTGTCTCCC
ATGGGAACCGGGGAGATACCGTTACTCCCGACGCCCACCGCCCTACCGAGGACAGAGGG
- 301 ArgGlySerArgProSerTrpGlyProThrAspProArgArgArgSerArgAsnLeuGly
CGTGGCTCTCGGCCTAGCTGGGGCCCCACAGACCCCGGCGTAGGTGCGCGCAATTTGGGT
GCACCGAGAGCCGGATCGACCCCGGGGTGTCTGGGGGCCGCATCCAGCGCTTAAACCCA
- 361 LysValIleAspThrLeuThrCysGlyPheAlaAspLeuMetGlyTyrIleProLeuVal
AAGGTCATCGATACCCCTTACGTGCGGCTTCGCGACCTCATGGGGTACATACCGCTCGTC
TTCCAGTAGCTATGGGAATGCACGCCGAAGCGGCTGGAGTACCCCATGTATGGCGAGCAG
- 421 GlyAlaProLeuGlyGlyAlaAlaArgAlaLeuAlaHisGlyValArgValLeuGluAsp
GGCGCCCCCTCTGGAGGCGCTGCCAGGGCCCTGGCGCATGGCGTCCGGGTCTGGAAGAC
CCGCGGGGAGAACCTCCGCGACGTTCCCGGACCGGTACCGCAGGCCCAAGACCTTCTG
- Thr
- 481 GlyValAsnTyrAlaThrGlyAsnLeuProGlyCysSerPheSerIlePheLeuLeuAla
GGCGTGAACATGCAACAGGGAACCTTCTGTTGCTCTTCTCTATCTTCTTCTGGCC
CCGCACTTGATACGTTGTCCCTTGAAGGACCAACGAGAAAGAGATAGAAGGAAGACCGG
- 541 LeuLeuSerCysLeuThrValProAlaSerAlaTyrGlnValArgAsnSerThrGlyLeu
CTGCTCTCTGCTTGACTGTGCGCGCTTCGGCCTACCAAGTGCAGCACTCCACGGGGCTT
GACGAGAGAACGAACTGACACGGGCGAAGCCGGATGGTTACGCGTTGAGGTGCCCCGAA
- 601 TyrHisValThrAsnAspCysProAsnSerSerIleValTyrGluAlaAlaAspAlaIle
TACCACGTCACCAATGATTGCCCTAACTCGAGTATTGTGTACGAGGCGGCCGATGCCATC
ATGGTGAGTGGTTACTAACGGGATTGAGCTCATAACACATGCTCCGCGGGCTACGGTAG
- 661 LeuHisThrProGlyCysValProCysValArgGluGlyAsnAlaSerArgCysTrpVal
CTGCACACTCCGGGGTGCCTCCCTTGCCTTCGTGAGGGCAACGCTCGAGGTGTGGGTG
GACGTGTGAGGCCCCACGCAGGGAACGCAAGCACTCCCGTTGCGGAGCTCCACAACCCAC

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FIG. 17-2

721 AlaMetThrProThrValAlaThrArgAspGlyLysLeuProAlaThrGlnLeuArgArg
GCGATGACCCCTACGGTGGCCACCAGGGATGGCAAAC TCCCGCGACGCAGCTTCGACGT
CGCTACTGGGGATGCCACCGTGGTCCCTACCGTTTGAGGGGCGCTGCGTCGAAGCTGCA

781 HisIleAspLeuLeuValGlySerAlaThrLeuCysSerAlaLeuTyrValGlyAspLeu
CACATCGATCTGCTTGTCTGGGAGCGCCACCCTCTGTTCCGGCCCTCTACGTGGGGGACCTA
GTGTAGCTAGACGAACAGCCCTCGCGGTGGGAGACAAGCCGGGAGATGCACCCCTGGAT

841 CysGlySerValPheLeuValGlyGlnLeuPheThrPheSerProArgArgHisTrpThr
TGCGGGTCTGTCTTTCTTGTCTGGCCAACTGTTACCTTCTCTCCAGGCGCCACTGGACG
ACGCCAGACAGAAAGAACAGCCGGTTGACAAGTGAAGAGAGGGTCCGCGGTGACCTGC

901 ThrGlnGlyCysAsnCysSerIleTyrProGlyHisIleThrGlyHisArgMetAlaTrp
ACGCAAGGTTGCAATTGCTCTATCTATCCCGCCATATAACGGGTACCCGATGGCATGG
TGCGTTCCAACGTTAACGAGATAGATAGGGCCGGTATATTGCCAGTGGCGTACCGTACC

961 Val
AspMetMetMetAsnTrpSerProThrThrAlaLeuValMetAlaGlnLeuLeuArgIle
GATATGATGATGAAC TGGTCCCTACGACGGCGTTGGTAATGGCTCAGCTGCTCCGGATC
CTATACTACTACTTGACCAGGGGATGCTGCCGCAACCATTACCGAGTCGACGAGGCCTAG

1021 ProGlnAlaIleLeuAspMetIleAlaGlyAlaHisTrpGlyValLeuAlaGlyIleAla
CCACAAGCCATCTTGGACATGATCGCTGGTGTCTCACTGGGGAGTCTGGCGGGCATAGCG
GGTGTTCGGTAGAACCTGTACTAGCGACCAGAGTGACCCCTCAGGACCGCCCGTATCGC

1081 TyrPheSerMetValGlyAsnTrpAlaLysValLeuValValLeuLeuPheAlaGly
TATTTCTCCATGGTGGGGAACTGGGCGAAGGTCCTGGTAGTGCTGCTGCTATTTGCCGGC
ATAAAGAGGTACCACCCCTTGACCCGCTTCCAGGACCATCACGACGACGATAAACGGCCG

1141 ValAspAlaGluThrHisValThrGlyGlySerAlaGlyHisThrValSerGlyPheVal
GTCGACGCGGAAACCCACGTCACCGGGGAAGTGCCGGCCACACTGTGTCTGGATTTGTT
CAGCTGCGCCTTTGGGTGCAGTGGCCCCCTTCACGGCCGGTGTGACACAGACCTAAACAA

1201 SerLeuLeuAlaProGlyAlaLysGlnAsnValGlnLeuIleAsnThrAsnGlySerTrp
AGCCTCTCTCGACCCAGGCGCCAAGCAGAACGTCAGCTGATCAACACCAACGGCAGTTGG
TCGGAGGAGCGTGGTCCGCGGTTCTGTCAGGTCGACTAGTTGTGGTTGCCGTCAACC

1261 HisLeuAsnSerThrAlaLeuAsnCysAsnAspSerLeuAsnThrGlyTrpLeuAlaGly
CACCTCAATAGCACGGCCCTGAAC TGAATGATAGCCTCAACACCGGCTGGTTGGCAGGG
GTGGAGTTATCGTGCCGGGACTTGACGTTACTATCGGAGTTGTGGCCGACCAACCGTCCC

1321 LeuPheTyrHisHisLysPheAsnSerSerGlyCysProGluArgLeuAlaSerCysArg
CTTTTCTATCACCACAAGTTCAACTCTTCAGGCTGTCTGAGAGGCTAGCCAGCTGCCGA
GAAAGATAGTGGTGTTCAGTTGAGAAGTCCGACAGGACTCTCCGATCGGTGACGGCT

1381 ProLeuThrAspPheAspGlnGlyTrpGlyProIleSerTyrAlaAsnGlySerGlyPro
CCCCTTACCGATTTTGACCAGGGCTGGGGCCCTATCAGTTATGCCAACGGAAGCGGCCCC
GGGGAATGGCTAAAACTGGTCCCGACCCCGGGATAGTCAATACGGTTGCCCTCGCCGGGG

1441 AspGlnArgProTyrCysTrpHisTyrProProLysProCysGlyIleValProAlaLys
GACCAGCGCCCTACTGCTGGCACTACCCCCAAAACCTTGCGGTATTGTGCCCCGGAAG
CTGGTCGCGGGGATGACGACCGTGATGGGGGTTTTGGAACGCCATAACACGGGCGCTTC

1501 SerValCysGlyProValTyrCysPheThrProSerProValValValGlyThrThrAsp
AGTGTGTGTGGTCCGGTATATTGCTTCACTCCCGCCCCGTGGTGGTGGGAACGACCGAC
TCACACACACAGGCCATATAACGAAGTGAGGGTCGGGGCACCACCACCTTGCTGGCTG

1561 ArgSerGlyAlaProThrTyrSerTrpGlyGluAsnAspThrAspValPheValLeuAsn
AGGTGCGGGCGCGCCACCTACAGCTGGGGTGAAAATGATACGGACGTCTTCGTCTCTAAC
TCCAGCCCGCGCGGGTGGATGTCGACCCCACTTTTACTATGCCTGCAGAAGCAGGAATTG

1621 AsnThrArgProProLeuGlyAsnTrpPheGlyCysThrTrpMetAsnSerThrGlyPhe
AATACCAGGCCACCGCTGGGCAATTGGTTCTGGTTGTACCTGGATGAAC TCAACTGGATTCT
TTATGGTCCGGTGGCGACCCGTTAACCAAGCCAACATGGACCTACTTGAGTTGACCTAAG

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FIG. 17-3

- 1681 ThrLysValCysGlyAlaProProCysValIleGlyGlyAlaGlyAsnAsnThrLeuHis
ACCAAAGTGTGCGGAGCGCTCCCTGTGTGTCATCGGAGGGGCGGGCAACAACCCCTGCAC
TGGTTTCACACGCCTCGCGGAGGAACACAGTAGCCTCCCCGCCCCGTTGTTGTGGGACGTG
- 1741 CysProThrAspCysPheArgLysHisProAspAlaThrTyrSerArgCysGlySerGly
TGCCCCACTGATTGCTTCCGCAAGCATCCGGACGCCACATACTCTCGGTGCGGCTCCGGT
ACGGGGTGACTAACGAAGGCGTTCGTAGGCCTGCGGTGTATGAGAGCCACGCCGAGGCCA
- Leu
- 1801 ProTrpIleThrProArgCysLeuValAspTyrProTyrArgLeuTrpHisTyrProCys
CCCTGGATCACACCCAGGTGCCTGGTTCGACTACCCGTATAGGCTTTGGCATTATCCTTGT
GGGACCTAGTGTGGGTCCACGGACCAGCTGATGGGCATATCCGAAACCGTAATAGGAACA
- 1861 ThrIleAsnTyrThrIlePheLysIleArgMetTyrValGlyGlyValGluHisArgLeu
ACCATCAACTACCCATATTTAAATCAGGATGTACGTGGGAGGGGTGCAACACAGGCTG
TGGTAGTTGATGTGGTATAAATTTAGTCTACATGCACCCTCCCCAGCTTGTGTCCGAC
- 1921 GluAlaAlaCysAsnTrpThrArgGlyGluArgCysAspLeuGluAspArgAspArgSer
GAAGCTGCCTGCAACTGGACGCGGGGCGAACGTTGCGATCTGGAAGACAGGGACAGGTCC
CTTCGACGGACGTTGACCTGCGCCCCGCTTGCAACGCTAGACCTTCTGTCCCTGTCCAGG
- 1981 GluLeuSerProLeuLeuLeuThrThrThrGlnTrpGlnValLeuProCysSerPheThr
GAGCTCAGCCCGTTACTGCTGACCACTACACAGTGGCAGGTCCCTCCCGTGTTCCTTCACA
CTCGAGTCGGGCAATGACGACTGGTGTGTGTACCGTCCAGGAGGGCACAAGGAAGTGT
- 2041 ThrLeuProAlaLeuSerThrGlyLeuIleHisLeuHisGlnAsnIleValAspValGln
ACCCTACGACCTTGTCCACCGGCCCTCATCCACCTCCACCAGAACATTGTGGACGTGCAG
TGGGATGGTCGGAACAGGTGGCCGGAGTAGGTGGAGGTGGTCTTGTAACACCTGCACGTC
- 2101 TyrLeuTyrGlyValGlySerSerIleAlaSerTrpAlaIleLysTrpGluTyrValVal
TACTGTACGGGGTGGGGTCAAGCATCGCGTCTGGGCCATTAAAGTGGGAGTACGTCTGTT
ATGAACATGCCCCACCCAGTTCGTAGCGCAGGACCCGGTAATTCACCCTCATGCAGCAA
- 2161 LeuLeuPheLeuLeuLeuAlaAspAlaArgValCysSerCysLeuTrpMetMetLeuLeu
CTCCTGTTCTTCTGCTTGCAGACGCGCGCTCTGCTCCTGCTTGTGGATGATGCTACTC
GAGGACAAGGAAGACGAACGTCTGCGCGCGCAGACGAGGACGAACACCTACTACGATGAG
- 2221 IleSerGlnAlaGluAlaAlaLeuGluAsnLeuValIleLeuAsnAlaAlaSerLeuAla
ATATCCCAAGCGGAGGCGGCTTTGGAGAACCTCGTAATACTTAATGCAGCATCCCTGGCC
TATAGGGTTTCGCTCCGCCGAAACCTCTTGAGCATTATGAATTACGTCTAGGGACCGG
- 2281 GlyThrHisGlyLeuValSerPheLeuValPhePheCysPheAlaTrpTyrLeuLysGly
GGGACGCACGGTCTTGTATCCTTCCTCGTGTCTTCTGCTTTGCATGGTATTGAAGGGT
CCCTGCGTGCCAGAACATAGGAAGGAGCACAAGAAGACGAAACGTACCATAAACTTCCCA
- 2341 LysTrpValProGlyAlaValTyrThrPheTyrGlyMetTrpProLeuLeuLeuLeuLeu
AAGTGGGTGCCCGGAGCGGTCTACACCTTCTACGGGATGTGGCCTCTCCTCCTGCTCTG
TTCACCCACGGGCCTCGCCAGATGTGGAAGATGCCCTACACCGGAGAGGAGGACGAGGAC
- 2401 LeuAlaLeuProGlnArgAlaTyrAlaLeuAspThrGluValAlaAlaSerCysGlyGly
TTGGCGTTGCCCCAGCGGGCGTACGCGCTGGACACGGAGGTGGCCGCGTCTGTGGCGGT
AACCACAACGGGGTCCCCGCATGCGCGACCTGTGCTCCACCGGCGCAGCACACGCCA
- 2461 ValValLeuValGlyLeuMetAlaLeuThrLeuSerProTyrTyrLysArgTyrIleSer
GTTGTTCCTCGTGGGTGATGGCGCTGACTCTGTACCATATTACAAGCGCTATATCAGC
CAACAAGAGCAGCCCACTACCGCGACTGAGACAGTGGTATAATGTTCCGATATAGTCG
- Asn
- 2521 TrpCysLeuTrpTrpLeuGlnTyrPheLeuThrArgValGluAlaGlnLeuHisValTrp
TGGTGCTTGTGGTGGCTTCAGTATTTTCTGACCAGAGTGGAAGCGCAACTGCACGTGTGG
ACCACGAACACCACCGAAGTCATAAAAGACTGGTCTCACCTTCGCGTTGACGTGCACACC
- 2581 IleProProLeuAsnValArgGlyGlyArgAspAlaValIleLeuLeuMetCysAlaVal
ATTCCCCCCTCAACGTCCGAGGGGGGCGGACGCGTCATCTTACTCATGTGTGCTGTA
TAAGGGGGGGAGTTGCAGGCTCCCCCGCGCTGCGGCAGTAGAATGAGTACACACGACAT

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FIG. 17-4

- 2641 HisProThrLeuValPheAspIleThrLysLeuLeuLeuAlaValPheGlyProLeuTrp
CACCCGACTCTGGTATTGACATCACCAATTGCTGCTGGCCGTCTTCGGACCCCTTTGG
GTGGGCTGAGACCATAAACTGTAGTGGTTTAACGACGACCGGCAGAAGCCTGGGGAAACC
- 2701 IleLeuGlnAlaSerLeuLeuLysValProTyrPheValArgValGlnGlyLeuLeuArg
ATTCTTCAAGCCAGTTTGCTTAAAGTACCCCTACTTTGTGCGCGTCCAAGGCCTTCTCCGG
TAAGAAGTTCGGTCAAACGAATTTTCATGGGATGAAACACGCGCAGGTTCCGGAAGAGGCC
- 2761 PheCysAlaLeuAlaArgLysMetIleGlyGlyHisTyrValGlnMetValIleIleLys
TTCTGCGCGTTAGCGCGGAAGATGATCGGAGGCCATTACGTGCAAATGGTCATCATTAAG
AAGACGCGCAATCGCGCCTTCTACTAGCCTCCGGAATGCACGTTTACCAGTAGTAATTC
- 2821 LeuGlyAlaLeuThrGlyThrTyrValTyrAsnHisLeuThrProLeuArgAspTrpAla
TTAGGGGCGCTTACTGGCACCTATGTTTATAACCATCTCACTCCTTTCGGGACTGGGCG
AATCCCCGGAATGACCGTGGATACAAATATTGGTAGAGTGAGGAGAAGCCCTGACCCGC
- 2881 HisAsnGlyLeuArgAspLeuAlaValAlaValGluProValValPheSerGlnMetGlu
CACAACGGCTTGCGAGATCTGGCCGTGGCTGTAGAGCCAGTCGTCTTCTCCCAAATGGAG
GTGTTGCCGAACGCTCTAGACCGGCACCGACATCTCGGTACGAGAAGAGGGTTTACCTC
- 2941 ThrLysLeuIleThrTrpGlyAlaAspThrAlaAlaCysGlyAspIleIleAsnGlyLeu
ACCAAGCTCATCAGTGGGGGGCAGATACCGCCGCGTGGGTGACATCATCAACGGCTTG
TGGTTCGAGTAGTGACCCCCCGTCTATGGCGGCGCACGCCACTGTAGTAGTTGCCGAAC
- 3001 ProValSerAlaArgArgGlyArgGluIleLeuLeuGlyProAlaAspGlyMetValSer
CCTGTTTTCCGCCCGCAGGGGCGGGAGATACTGCTCGGGCCAGCCGATGGAATGGTCTCC
GGACAAAGGCGGGCGTCCCCGGCCCTCTATGACGAGCCCGTCCGGCTACCTTACCAGAGG
- 3061 LysGlyTrpArgLeuLeuAlaProIleThrAlaTyrAlaGlnGlnThrArgGlyLeuLeu
AAGGGGTGGAGTTGCTGGCGCCCATCACGGCGTACGCCAGCAGACAAGGGGCCTCCTA
TTCCCCACCTCCAACGACCGCGGTAGTCCCGCATGCGGGTCTGTCTGTTCCCCGGAGGAT
- 3121 GlyCysIleIleThrSerLeuThrGlyArgAspLysAsnGlnValGluGlyGluValGln
GGGTGCATAATCACCAGCCTAACTGGCCGGGACAAAAACCAAGTGGAGGGTGAGGTCCAG
CCCACGTATTAGTGGTTCGGATTGACCGGCCCTGTTTTTGGTTACCTCCCACTCCAGGTC
- 3181 IleValSerThrAlaAlaGlnThrPheLeuAlaThrCysIleAsnGlyValCysTrpThr
ATTGTGTCAACTGCTGCCCAAACCTTCTGGCAACGTGCATCAATGGGGTGTGCTGGACT
TAACACAGTTGACGACGGGTTTGAAGGACCGTTGCACGTAGTTACCCACACGACCTGA
- 3241 ValTyrHisGlyAlaGlyThrArgThrIleAlaSerProLysGlyProValIleGlnMet
GTCTACCACGGGGCCGGAACGAGGACCATCGCGTCACCAAGGGTCTGTGCATCCAGATG
CAGATGGTGGCCCGCCTTGCTCCTGGTAGCGCAGTGGGTTCCAGGACAGTAGGTCTAC
- 3301 TyrThrAsnValAspGlnAspLeuValGlyTrpProAlaProGlnGlySerArgSerLeu
TATACCAATGTAGACCAAGACCTTGTGGGCTGGCCCGCTCCGCAAGGTAGCCGCTCATTG
ATATGGTTACATCTGGTTCTGGAACACCCGACCGGGCGAGGCGTTCCATCGGCGAGTAAC
- 3361 ThrProCysThrCysGlySerSerAspLeuTyrLeuValThrArgHisAlaAspValIle
ACACCCTGCACTTGGCGCTCCTCGGACCTTTACCTGGTACGAGGCACGCCGATGTCATT
TGTGGGACGTGAACGCCGAGGAGCCTGGAATGGACCAAGTGTCTCCGTGCGGCTACAGTAA
- 3421 ProValArgArgArgGlyAspSerArgGlySerLeuLeuSerProArgProIleSerTyr
CCCGTGGCGCCGGCGGGGTGATAGCAGGGGCAGCCTGCTGTGCCCCGGCCCCATTTCCTAC
GGGCACGCGCGCCGCCCACTATCGTCCCCGTGCGACGACAGCGGGGCCGGGTAAAGGATG
- 3481 LeuLysGlySerSerGlyGlyProLeuLeuCysProAlaGlyHisAlaValGlyIlePhe
TTGAAAGGCTCCTCGGGGGGTCCGCTGTTGTGCCCCGCGGGGCACGCCGTGGGCATATTT
AATTTCCGAGGAGCCCCCAGGCGACAACACGGGGCGCCCCGTGCGGCACCCGTATAAA
- 3541 ArgAlaAlaValCysThrArgGlyValAlaLysAlaValAspPheIleProValGluAsn
AGGGCCGCGGTGTGCACCCGTGGAGTGGCTAAGGCGGTGGACTTTATCCCTGTGGAGAAC
TCCCCGCCACACAGTGGGCACCTACCGATTCCGCCACCTGAAATAGGGACACCTCTTG

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FIG. 17-5

- 3601 LeuGluThrThrMetArgSerProValPheThrAspAsnSerSerProProValValPro
CTAGAGACAACCATGAGGTCCCCGGTGTTCACGGATAACTCCTCTCCACCAGTAGTGCC
GATCTCTGTTGGTACTCCAGGGGCCACAAGTGCCTATTGAGGAGAGGTGGTCATCACGGG
- 3661 GlnSerPheGlnValAlaHisLeuHisAlaProThrGlySerGlyLysSerThrLysVal
CAGAGCTTCCAGGTGGCTCACCTCCATGCTCCCACAGGCAGCGGCAAAAGCACCAAGGTC
GTCTCGAAGGTCCACCGAGTGGAGGTACGAGGGTGTCCGTCGCCGTTTTCGTGGTTCCAG
- 3721 ProAlaAlaTyrAlaAlaGlnGlyTyrLysValLeuValLeuAsnProSerValAlaAla
CCGGCTGCATATGCAGCTCAGGGCTATAAGGTGCTAGTACTCAACCCCTCTGTTGCTGCA
GGCCGACGTATACGTCGAGTCCCAGATATTCCACGATCATGAGTTGGGGAGACAACGACGT
- 3781 ThrLeuGlyPheGlyAlaTyrMetSerLysAlaHisGlyIleAspProAsnIleArgThr
ACACTGGGCTTTGGTGCTTACATGTCCAAGGCTCATGGGATCGATCCTAACATCAGGACC
TGTGACCCGAAACCACGAATGTACAGGTTCCGAGTACCCTAGCTAGGATTGTAGTCTGG
- 3841 GlyValArgThrIleThrThrGlySerProIleThrTyrSerThrTyrGlyLysPheLeu
GGGGTGAGAACAATTACCACTGGCAGCCCCATCACGTACTCCACCTACGGCAAGTTCCTT
CCCCACTCTTGTTAATGGTGACCGTCGGGGTAGTGCATGAGGTGGATGCCGTTCAAGGAA
- 3901 AlaAspGlyGlyCysSerGlyGlyAlaTyrAspIleIleIleCysAspGluCysHisSer
GCCGACGGCGGGTGCTCGGGGGGCGCTTATGACATAATAATTTGTGACGAGTGCCACTCC
CGGCTGCCGCCACGAGCCCCCGCGAATACTGTATTATTAAACACTGCTCACGGTGAGG
- 3961 ThrAspAlaThrSerIleLeuGlyIleGlyThrValLeuAspGlnAlaGluThrAlaGly
ACGGATGCCACATCCATCTTGGGCATCGGCACTGTCTTGACCAAGCAGAGACTGCGGGG
TGCTTACGGTGTAGGTAGAACCCGTAGCCGTGACAGGAAGTGGTTCGTCTCTGACGCCCC
- 4021 AlaArgLeuValValLeuAlaThrAlaThrProProGlySerValThrValProHisPro
GCGAGACTGGTTGTGCTCGCCACCGCCACCCCTCCGGGCTCCGTCATGTGCCCCATCCC
CGCTCTGACCAACACGAGCGGTGGCGGTGGGGAGGCCCGAGGCAGTGACACGGGGTAGGG
- 4081 AsnIleGluGluValAlaLeuSerThrThrGlyGluIleProPheTyrGlyLysAlaIle
AACATCGAGGAGGTTGCTCTGTCCACCACCGGAGAGATCCCTTTTTACGGCAAGGCTATC
TTGTAGCTCCTCCAACGAGACAGGTGGTGGCCTCTCTAGGGAAAATGCCGTTCCGATAG
- 4141 ProLeuGluValIleLysGlyGlyArgHisLeuIlePheCysHisSerLysLysLysCys
CCCCTCGAAGTAATCAAGGGGGGAGACATCTCATCTTCTGTCAATTCAAAGAAGAAGTGC
GGGGAGCTTCATTAGTTCCCCCCTCTGTAGAGTAGAAGACAGTAAGTTTCTTCTTCACG
- 4201 AspGluLeuAlaAlaLysLeuValAlaLeuGlyIleAsnAlaValAlaTyrTyrArgGly
GACGAACCTCGCCGAAAGCTGGTTCGCATTGGGCATCAATGCCGTGGCCTACTACCGCGGT
CTGCTTGAGCGGCGTTTCGACCAGCGTAACCCGTAGTTACGGCACCGGATGATGGCGCCA
- 4261 LeuAspValSerValIleProThrSerGlyAspValValValValAlaThrAspAlaLeu
CTTGACGTGTCCGTATCCCGACCGCGGATGTTGTCTGTCGTGGCAACCGATGCCCTC
GAACGTCACAGGCAGTAGGGCTGGTCCGCGCTACAACAGCAGCACCGTTGGCTACGGGAG
- 4321 MetThrGlyTyrThrGlyAspPheAspSerValIleAspCysAsnThrCysValThrGln
ATGACCGGCTATACCGGCGACTTCGACTCGGTGATAGACTGCAATACGTGTGTACCCAG
TACTGGCCGATATGGCCGCTGAAGCTGAGCCACTATCTGACGTTATGCACACAGTGGGTC
- 4381 ThrValAspPheSerLeuAspProThrPheThrIleGluThrIleThrLeuProGlnAsp
ACAGTCGATTTTCAGCCTTGACCTTACCTTACCATTTGAGACAATCAGCTCCCCCAGGAT
TGTACAGCTAAAGTCGGAACCTGGGATGGAAGTGGTAACTCTGTAGTGGAGGGGGTCTTA
- 4441 AlaValSerArgThrGlnArgArgGlyArgThrGlyArgGlyLysProGlyIleTyrArg
GCTGTCTCCCGCACTCAACGTCGGGGCAGGACTGGCAGGGGGAAGCCAGGCATCTACAGA
CGACAGAGGGCGTGAGTTGCAGCCCCGTCTGACCGTCCCCCTTCGGTCCGTAGATGTCT
- 4501 PheValAlaProGlyGluArgProSerGlyMetPheAspSerSerValLeuCysGluCys
TTTGTGGCACCGGGGGAGCGCCCTCCGGCATGTTGACTCGTCCGTCCTCTGTGAGTGC
AAACACCGTGGCCCCCTCGCGGGGAGGCCGTACAAGCTGAGCAGGCAGGAGACACTCAGC

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FIG. 17-6

4561 TyrAspAlaGlyCysAlaTrpTyrGluLeuThrProAlaGluThrThrValArgLeuArg
TATGACGCAGGCTGTGCTTGGTATGAGCTCACGCCCCGAGACTACAGTTAGGCTACGA
ATACTGCGTCCGACACGAACATACTCGAGTGCGGGCGGCTCTGATGTCAATCCGATGCT

4621 AlaTyrMetAsnThrProGlyLeuProValCysGlnAspHisLeuGluPheTrpGluGly
GCGTACATGAACACCCCGGGGCTTCCCGTGTGCCAGGACCATCTTGAAATTTGGGAGGGC
CGCATGTACTTGTGGGGCCCCGAAGGACACACGGTCCTGGTAGAACTTAAACCTCCCC

4681 ValPheThrGlyLeuThrHisIleAspAlaHisPheLeuSerGlnThrLysGlnSerGly
GTCTTTACAGGCCTCACTCATATAGATGCCACTTTCTATCCCAGACAAAGCAGAGTGGG
CAGAAATGTCCGGAGTGAGTATATCTACGGGTGAAAGATAGGGTCTGTTTCGTCTCACC

4741 GluAsnLeuProTyrLeuValAlaTyrGlnAlaThrValCysAlaArgAlaGlnAlaPro
GAGAACCTTCCTTACCTGGTAGCGTACCAAGCCACCGTGTGCGCTAGGGCTCAAGCCCCCT
CTCTTGGAAGGAATGGACCATCGCATGGTTCGGTGGCACACCGGATCCCGAGTTCGGGGA

4801 ProProSerTrpAspGlnMetTrpLysCysLeuIleArgLeuLysProThrLeuHisGly
CCCCATCGTGGGACCAGATGTGGAAGTGTGTTGATTGCGCTCAAGCCCACCTCCATGGG
GGGGGTAGCACCTGGTCTACACCTTCAAACTAAGCGGAGTTCGGGTGGGAGGTACCC

4861 ProThrProLeuLeuTyrArgLeuGlyAlaValGlnAsnGluIleThrLeuThrHisPro
CCAACACCCCTGCTATACAGACTGGGCGCTGTTTCAAGATGAAATCACCTGACGCACCCA
GGTGTGGGGACGATATGTGTGACCCGCGACAAGTCTTACTTTAGTGGGACTGCGTGGGT

4921 ValThrLysTyrIleMetThrCysMetSerAlaAspLeuGluValValThrSerThrTrp
GTCACCAAATACATCATGACATGCATGTGCGCCGACCTGGAGGTGCTCACGAGCACCTGG
CAGTGGTTTATGTAGTACTGTACGTACAGCCGGCTGGACCTCCAGCAGTGTCTCGTGACC

4981 ValLeuValGlyGlyValLeuAlaAlaLeuAlaAlaTyrCysLeuSerThrGlyCysVal
GTGCTCGTTGGCGGCGTCTGGCTGCTTTGGCCGCGTATTGCCTGTCAACAGGCTGCGTG
CACGAGCAACCGCCGAGGACCGACGAAACCGGCGCATAACGGACAGTTGTCCGACGCAC

5041 ValIleValGlyArgValValLeuSerGlyLysProAlaIleIleProAspArgGluVal
GTCATAGTGGGCAGGGTCTGCTTGTCCGGGAAGCCGGCAATCATACTGACAGGGAAGTC
CAGTATCACCCGTCCCAGCAGAACAGGCCCTTCGGCCGTTAGTATGGACTGTCCCTTCAG

5101 LeuTyrArgGluPheAspGluMetGluGluCysSerGlnHisLeuProTyrIleGluGln
CTCTACCGAGAGTTCGATGAGATGGAAGAGTGCTCTCAGCACTTACCGTACATCGAGCAA
GAGATGGCTCTCAAGCTACTCTACCTTCTCACGAGAGTCTGTAATGGCATGTAGCTCGTT

5161 GlyMetMetLeuAlaGluGlnPheLysGlnLysAlaLeuGlyLeuLeuGlnThrAlaSer
GGGATGATGCTCGCCGAGCAGTTCAAGCAGAAGGCCCTCGGCCTCCTGCAGACCGCGTCC
CCCTACTACGAGCGGCTCGTCAAGTTCTGCTTCCGGGAGCCGGAGGACGTCTGGCGCAGG

5221 ArgGlnAlaGluValIleAlaProAlaValGlnThrAsnTrpGlnLysLeuGluThrPhe
CGTCAGGCAGAGGTTATCGCCCCCTGCTGTCCAGACCAACTGGCAAAAACCGAGACCTTC
GCAGTCCGTCTCCAATAGCGGGGACGACAGGTCTGGTTGACCGTTTTTGGAGCTCTGGAAG

5281 TrpAlaLysHisMetTrpAsnPheIleSerGlyIleGlnTyrLeuAlaGlyLeuSerThr
TGGGCGAAGCATATGTGGAACCTTCATCAGTGGGATAACAATACTTGGCGGGCTTGTCACG
ACCCGCTTCGTATACACCTTGAAGTAGTCACCCTATGTTATGAACCGCCCGAACAGTTGC

5341 LeuProGlyAsnProAlaIleAlaSerLeuMetAlaPheThrAlaAlaValThrSerPro
CTGCCTGGTAACCCCGCCATTGCTTCATTGATGGCTTTTACAGCTGCTGTACCAGCCCA
GACGGACCATTTGGGGCGGTAACGAAGTAACACCGAAAATGTCGACGACAGTGGTCCGGT

5401 LeuThrThrSerGlnThrLeuLeuPheAsnIleLeuGlyGlyTrpValAlaAlaGlnLeu
CTAACCCTAGCCAAACCTCCTCTTCAACATATTGGGGGGGTGGGTGGCTGCCAGCTC
GATTGGTGATCGGTTTGGGAGGAGAAGTTGTATAACCCCCCACCACCGAGGGTTCGAG

5461 AlaAlaProGlyAlaAlaThrAlaPheValGlyAlaGlyLeuAlaGlyAlaAlaIleGly
GCGGCCCCCGGTGCCGCTACTGCCCTTTGTGGGCGCTGGCTTAGCTGGCGCCGCCATCGGC
CGGCGGGGGCCACGGCGATGACGGAAACACCCGCGACCGAATCGACCGCGGCGGTAGCCG

SerValGlyLeuGlyLysValLeuIleAspIleLeuAlaGlyTyrGlyAlaGlyValAla

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FIG. 17-7

- 5521 AGTGTGGACTGGGGAAGGTCTCATAGACATCTTGCAGGGTATGGCGCGGGCGTGGCG
TCACAACCTGACCCCTTCCAGGAGTATCTGTAGGAACGTCCCATACCGCGCCCGCACCGC
- 5581 GlyAlaLeuValAlaPheLysIleMetSerGlyGluValProSerThrGluAspLeuVal Gly
GGAGCTCTTGTGGCATTCAAGATCATGAGCGGTGAGGTCCCTCCACGGAGGACCTGGTC
CCTCGAGAACACCGTAAGTTCTAGTACTCGCCACTCCAGGGGAGGTGCCTCCTGGACCAG
- 5641 AsnLeuLeuProAlaIleLeuSerProGlyAlaLeuValValGlyValValCysAlaAla
AATCTACTGCCCCCATCTCTCGCCCGGAGCCCTCGTAGTCGGCGTGGTCTGTGCAGCA
TTAGATGACGGGCGGTAGGAGAGCGGGCCTCGGGAGCATCAGCCGCACCAGACACGTCGT
- 5701 IleLeuArgArgHisValGlyProGlyGluGlyAlaValGlnTrpMetAsnArgLeuIle
ATACTGCGCCGGCAGCTTGGCCCGGGCGAGGGGCGAGTGCAGTGGATGAACCGGCTGATA
TATGACGCGGCCGTGCAACCGGGCCCGCTCCCCCGTCACGTACCTACTTGGCCGACTAT
- 5761 AlaPheAlaSerArgGlyAsnHisValSerProThrHisTyrValProGluSerAspAla
GCCTTCGCTCCCGGGGAACCATGTTTCCCCACGCACTACGTGCCGGAGAGCGATGCA
CGAAGCGGAGGGCCCCCTTGGTACAAAGGGGTGCGTGATGCACGGCCTCTCGCTACGT
- 5821 HisCys
AlaAlaArgValThrAlaIleLeuSerSerLeuThrValThrGlnLeuLeuArgArgLeu
GCTGCCCCGCTCACTGCCATACTCAGCAGCCTCACTGTAACCCAGCTCCTGAGGCGACTG
CGACGGGCGCAGTGACGGTATGAGTCGTGGAGTGACATTGGGTGAGGACTCCGCTGAC
- 5881 HisGlnTrpIleSerSerGluCysThrThrProCysSerGlySerTrpLeuArgAspIle
CACCAGTGGATAAGCTCGGAGTGTAACCTCCATGCTCCGGTTCCTGGCTAAGGGACATC
GTGGTCACCTATTGAGCCTCACATGGTGAGGTACGAGGCCAAGGACCGATTCCCTGTAG
- 5941 TrpAspTrpIleCysGluValLeuSerAspPheLysThrTrpLeuLysAlaLysLeuMet
TGGGACTGGATATGCGAGGTGTTGAGCGACTTTAAGACCTGGCTAAAAGCTAAGCTCATG
ACCCTGACCTATACGCTCCACAACCTCGCTGAAATTCTGGACCGATTTTCGATTGAGTAC
- 6001 ProGlnLeuProGlyIleProPheValSerCysGlnArgGlyTyrLysGlyValTrpArg
CCACAGCTGCCTGGGATCCCTTTGTGTCTGCCAGCGCGGGTATAAGGGGGTCTGGCGA
GGTGTGACGACCGACCTAGGGGAAACACAGGACGGTCGCGCCCATATCCCCCAGACCGCT
- 6061 Gly
ValAspGlyIleMetHisThrArgCysHisCysGlyAlaGluIleThrGlyHisValLys
GTGGACGGCATCATGCACACTCGCTGCCACTGTGGAGCTGAGATCACTGGACATGTCAA
CACCTGCCGTAGTACGTGTGAGCGACGGTGACACCTCGACTCTAGTGACCTGTACAGTTT
- 6121 AsnGlyThrMetArgIleValGlyProArgThrCysArgAsnMetTrpSerGlyThrPhe
AACGGGACGATGAGGATCGTCCGTCTAGGACCTGCAGGAACATGTGGAGTGGGACCTTC
TTGCCCTGCTACTCCTAGCAGCCAGGATCCTGGACGTCTTGTACACCTCACCTTGAAG
- 6181 ProIleAsnAlaTyrThrThrGlyProCysThrProLeuProAlaProAsnTyrThrPhe
CCCATTAATGCCTACACCACGGGCCCCGTGTACCCCCCTTCTGCGCCGAACCTACAGTTT
GGTAATTACGGATGTGGTGCCCGGGGACATGGGGGGAAGGACGCGGCTTGATGTGCAAG
- 6241 AlaLeuTrpArgValSerAlaGluGluTyrValGluIleArgGlnValGlyAspPheHis
GCGCTATGGAGGGTGTCTGCAGAGGAATATGTGGAGATAAGGCAGGTGGGGGACTTCCAC
CGCGATACCTCCACAGACGTCTCCTTATACACCTCTATTCCGTCCACCCCTGAAGGTG
- 6301 TyrValThrGlyMetThrThrAspAsnLeuLysCysProCysGlnValProSerProGlu
TACGTGACGGGTATGACTACTGACAATCTCAAATGCCCGTGCCAGGTCCCATCGCCGAA
ATGCACTGCCCATACTGATGACTGTTAGAGTTTACGGGCACGGTCCAGGGTAGCGGGCTT
- 6361 PhePheThrGluLeuAspGlyValArgLeuHisArgPheAlaProProCysLysProLeu
TTTTTTCACAGAATTGGACGGGGTGCCTACATAGGTTTGGCGCCCCCTGCAAGCCCTTG
AAAAAGTGTCTTAACCTGCCCCACGCGGATGTATCCAAACGCGGGGGGACGTTTCGGGAAC
- 6421 LeuArgGluGluValSerPheArgValGlyLeuHisGluTyrProValGlySerGlnLeu
CTGCGGGAGGAGGTATCATTCAGAGTAGGACTCCACGAATACCCGGTAGGGTCGCAATTA
GACGCCCTCCTCATAGTAAGTCTCATCCTGAGGTGCTTATGGGCCATCCAGCGTTAAT

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FIG. 17-8

- 6481 ProCysGluProGluProAspValAlaValLeuThrSerMetLeuThrAspProSerHis
CCTTGCGAGCCCGAACCGGACGTGGCCGTGTTGACGTCCATGCTCACTGATCCCTCCCAT
GGAACGCTCGGGCTTGGCCTGCACCGGCACAACTGCAGGTACGAGTGACTAGGGAGGGTA
- 6541 IleThrAlaGluAlaAlaGlyArgArgLeuAlaArgGlySerProProSerValAlaSer
ATAACAGCAGAGGCGGCGGGCGAAGGTTGGCGAGGGGATCACCCCCCTCTGTGGCCAGC
TATTGTGCTCTCCGCCGGCCCGCTTCCAACCGCTCCCCTAGTGGGGGGAGACACCGGTCG
- 6601 SerSerAlaSerGlnLeuSerAlaProSerLeuLysAlaThrCysThrAlaAsnHisAsp
TCCTCGGCTAGCCAGCTATCCGCTCCATCTCTCAAGGCAACTTGCACCGCTAACCATGAC
AGGAGCCGATCGGTTCGATAGGCGAGGTAGAGAGTTCCGTTGAACGTGGCGATTGGTACTG
- 6661 SerProAspAlaGluLeuIleGluAlaAsnLeuLeuTrpArgGlnGluMetGlyGlyAsn
TCCCCTGATGCTGAGCTCATAGAGGCCAACCTCCTATGGAGGCAGGAGATGGGCGGCAAC
AGGGGACTACGACTCGAGTATCTCCGTTGGAGGATACCTCCGTCTCTACCCGCCGTTG
- 6721 IleThrArgValGluSerGluAsnLysValValIleLeuAspSerPheAspProLeuVal
ATCACCAGGGTTGAGTCAGAAAACAAAGTGGTGATTCTGGACTCCTTCGATCCGCTTGTC
TAGTGGTCCCAACTCAGTCTTTTGTTCACCACTAAGACCTGAGGAAGCTAGGCGAACAC
- 6781 AlaGluGluAspGluArgGluIleSerValProAlaGluIleLeuArgLysSerArgArg
GCGGAGGAGGACGAGCGGGAGATCTCCGTACCCGCAGAAATCCTGCGGAAGTCTCGGAGA
CGCCTCCTCTGCTCGCCCTCTAGAGGCATGGGCGTCTTTAGGACGCCTTCAGAGCCTCT
- 6841 PheAlaGlnAlaLeuProValTrpAlaArgProAspTyrAsnProProLeuValGluThr
TTCGCCAGGCCCTGCCCGTTTGGGCGCGGCCGACTATAACCCCCGCTAGTGAGACG
AAGCGGGTCCGGGACGGGCAAACCCGCGCGGCCCTGATATTGGGGGGCGATCACCTCTGC
- 6901 TrpLysLysProAspTyrGluProProValValHisGlyCysProLeuProProProLys
TGGAAAAAGCCCGACTACGAACCACTGTGGTCCATGGCTGTCCGCTTCCACCTCCAAAG
ACCTTTTTCGGGCTGATGCTTGGTGGACACCAGGTACCGACAGGCGAAGGTGGAGGTTTC
- 6961 SerProProValProProProArgLysLysArgThrValValLeuThrGluSerThrLeu
TCCCCTCCTGTGCCTCCGCCTCGGAAGAAGCGGACGGTGGTCCCTCACTGAATCAACCCTA
AGGGGAGGACACGGAGGCGGAGCCTTCTTCGCCTGCCACCAGGAGTGACTTAGTTGGGAT
- Ser
- 7021 SerThrAlaLeuAlaGluLeuAlaThrArgSerPheGlySerSerSerThrSerGlyIle
TCTACTGCCTTGGCCGAGCTCGCCACCAGAAGCTTTGGCAGCTCCTCAACTCCGGCATT
AGATGACGGAACCGGCTCGAGCGGTGGTCTTCGAAACCGTCGAGGAGTTGAAGCCGTAA
- 7081 ThrGlyAspAsnThrThrThrSerSerGluProAlaProSerGlyCysProProAspSer
ACGGGCGACAATACGACAACATCCTCTGAGCCCGCCCCCTTCTGGCTGCCCCCGACTCC
TGCCCGCTGTTATGCTGTTGTAGGAGACTCGGGCGGGGAAGACCGACGGGGGGCTGAGG
- PheAla
- 7141 AspAlaGluSerTyrSerSerMetProProLeuGluGlyGluProGlyAspProAspLeu
GACGCTGAGTCCTATTCTCCATGCCCCCTGGAGGGGGAGCCTGGGGATCCGGATCTT
CTGCGACTCAGGATAAGGAGGTACGGGGGGGACCTCCCCCTCGGACCCCTAGGCCTAGAA
- 7201 SerAspGlySerTrpSerThrValSerSerGluAlaAsnAlaGluAspValValCysCys
AGCGACGGGTCATGGTCAACGGTCAGTAGTGAGGCCAACGCGGAGGATGTCGTGTGCTGC
TCGCTGCCCAGTACCAGTTGCCAGTCATCACTCCGTTGCGCCTCCTACGACACAGACG
- 7261 SerMetSerTyrSerTrpThrGlyAlaLeuValThrProCysAlaAlaGluGluGlnLys
TCAATGTCTTACTCTTGGACAGGCGCACTCGTCACCCCGTGGCGCGCGGAAGAAGAGAA
AGTTACAGAATGAGAACCTGTCCGCGTGAGCAGTGGGGCACGCGGCGCCTTCTTGTCTTT
- 7321 LeuProIleAsnAlaLeuSerAsnSerLeuLeuArgHisHisAsnLeuValTyrSerThr
CTGCCCATCAATGCACTAAGCAACTCGTTGCTACGTACCCACAATTGGTGATTTCCACC
GACGGGTAGTTACGTGATTCGTTGAGCAACGATGCAGTGGTGTTAAACCACATAAGGTGG
- 7381 ThrSerArgSerAlaCysGlnArgGlnLysLysValThrPheAspArgLeuGlnValLeu
ACCTCACGCAGTGCTTGCCAAAGGCAGAAAGTCAATTTGACAGACTGCAAGTTCTG
TGGAGTGCGTCACGAACGGTTTCCGTCTTCTTTCAGTGTAACCTGTCTGACGTTCAAGAC

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FIG. 17-9

- 7441 AspSerHisTyrGlnAspValLeuLysGluValLysAlaAlaAlaSerLysValLysAla
GACAGCCATTACCAGGACGTACTCAAGGAGGTTAAAGCAGCGGCGTCAAAGTGAAGGCT
CTGTCCGTAATGGTCTGCATGAGTTCCTCCAATTTCTGTCGCCGAGTTTTCACTTCCGA
- Phe
- 7501 AsnLeuLeuSerValGluGluAlaCysSerLeuThrProProHisSerAlaLysSerLys
AACTTGCTATCCGTAGAGGAAGCTTGCAGCCTGACGCCCCCACTCAGCCAAATCCAAG
TTGAACGATAGGCATCTCTTCGAACGTCGGACTGCGGGGGTGTGAGTCGGTTTAGGTTT
- 7561 PheGlyTyrGlyAlaLysAspValArgCysHisAlaArgLysAlaValThrHisIleAsn
TTTGGTTATGGGGCAAAGACGTCCGTTGCCATGCCAGAAAGGCCGTAAACCCACATCAAC
AAACCAATACCCCGTTTTCTGCAGGCAACGGTACGGTCTTTCCGGCATTGGGTGTAGTTG
- 7621 SerValTrpLysAspLeuLeuGluAspAsnValThrProIleAspThrThrIleMetAla
TCCGTGTGGAAAGACCTTCTGGAAGACAATGTAACACCAATAGACACTACCATCATGGCT
AGGCACACCTTTCTGGAAGACCTTCTGTTACATTGTGGTTATCTGTGATGGTAGTACCGA
- 7681 LysAsnGluValPheCysValGlnProGluLysGlyGlyArgLysProAlaArgLeuIle
AAGAACGAGGTTTTCTGCGTTTCAGCCTGAGAAGGGGGTTCGTAAGCCAGCTCGTCTCATC
TTCTTGCTCCAAAAGACGCAAGTCGGACTCTTCCCCCAGCATTCGGTCGAGCAGAGTAG
- 7741 ValPheProAspLeuGlyValArgValCysGluLysMetAlaLeuTyrAspValValThr
GTGTTCCCCGATCTGGGCGTGGCGGTGTCGCAAAAGATGGCTTTGTACGACGTGGTTACA
CACAAGGGGCTAGACCCGCACGCGCACGCTTTTCTACCGAAACATGCTGCACCAATGT
- 7801 LysLeuProLeuAlaValMetGlySerSerTyrGlyPheGlnTyrSerProGlyGlnArg
AAGCTCCCCCTGGCCGTGATGGGAAGCTCCTACGGATTCCAATACTCACCAGGACAGCGG
TTCGAGGGGAACCGGCACTACCTTCGAGGATGCCTAAGGTTATGAGTGGTCTGTGCGC
- 7861 ValGluPheLeuValGlnAlaTrpLysSerLysLysThrProMetGlyPheSerTyrAsp
GTTGAATTCCTCGTGCAAGCGTGGAAGTCCAAGAAAACCCCAATGGGGTTCTCGTATGAT
CAACTTAAGGAGCACGTTTCGCACCTTCAGGTTCTTTTGGGGTTACCCCAAGAGCATACTA
- 7921 ThrArgCysPheAspSerThrValThrGluSerAspIleArgThrGluGluAlaIleTyr
ACCCGCTGCTTTGACTCCACAGTCACTGAGAGCGACATCCGTACGGAGGAGGCAATCTAC
TGGGCGACGAAACTGAGGTGTCAGTCACTCTCGCTGTAGGCATGCCTCCTCCGTTAGATG
- 7981 GlnCysCysAspLeuAspProGlnAlaArgValAlaIleLysSerLeuThrGluArgLeu
CAATGTTGTGACCTCGACCCCCAAGCCCGCGTGGCCATCAAGTCCCTCACCAGAGAGGCTT
GTTACAACACTGGAGCTGGGGGTTCCGGGCGACCGGTAGTTCAGGGAGTGGCTCTCCGAA
- Gly
- 8041 TyrValGlyGlyProLeuThrAsnSerArgGlyGluAsnCysGlyTyrArgArgCysArg
TATGTTGGGGGCCCTCTTACCAATTCAGGGGGGAGAACTGCGGCTATCGCAGGTGCCCG
ATACAACCCCGGGAGAAATGGTTAAGTTCCCCCTCTTGACGCCGATAGCGTCCACGGCG
- 8101 AlaSerGlyValLeuThrThrSerCysGlyAsnThrLeuThrCysTyrIleLysAlaArg
GCGAGCGGCGTACTGACAAGTACTGTTGGTAACACCCTCACTTGCTACATCAAGGCCCCG
CGCTCGCCGCATGACTGTTGATCGACACCATTGTGGGAGTGAACGATGTAGTTCCGGGCC
- 8161 AlaAlaCysArgAlaAlaGlyLeuGlnAspCysThrMetLeuValCysGlyAspAspLeu
GCAGCCTGTCGAGCCGCGAGGGCTCCAGGACTGCACCATGCTCGTGTGTGGCGACGACTTA
CGTCGGACAGCTCGGCGTCCCGAGGTCCTGACGTGGTACGAGCACACACCGCTGCTGAAT
- 8221 ValValIleCysGluSerAlaGlyValGlnGluAspAlaAlaSerLeuArgAlaPheThr
GTCGTTATCTGTGAAAGCGGGGGTCCAGGAGGACGCGGCGAGCCTGAGAGCCTTCAGG
CAGCAATAGACACTTTTCGCGCCCCCAGGTCTCTCGCGCGCTCGGACTCTCGGAAGTGC
- 8281 GluAlaMetThrArgTyrSerAlaProProGlyAspProProGlnProGluTyrAspLeu
GAGGCTATGACCAGGTACTCCGCCCCCCTGGGGACCCCCACAACCAGAAATACGACTTG
CTCCGATACTGGTCCATGAGGCGGGGGGACCCCTGGGGGGTGTGGTCTTATGCTGAAC
- 8341 GluLeuIleThrSerCysSerSerAsnValSerValAlaHisAspGlyAlaGlyLysArg
GAGCTCATAACATCATGCTCCTCCAACGTGTCAGTCGCCCACGACGGCGCTGGAAAGAGG
CTCGAGTATTGTAGTACGAGGAGGTTGCACAGTCAGCGGGTGTGCTGCCGCGACCTTTCTCC

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8401 ValTyrTyrLeuThrArgAspProThrThrProLeuAlaArgAlaAlaTrpGluThrAla
GTCTACTACCTCACCCGTGACCCTACAACCCCCCTCGCGAGAGCTGCGTGGGAGACAGCA
CAGATGATGGAGTGGGCACTGGGATGTTGGGGGGAGCGCTCTCGACGCACCCTCTGTCTGT

8461 ArgHisThrProValAsnSerTrpLeuGlyAsnIleIleMetPheAlaProThrLeuTrp
AGACACACTCCAGTCAATTCCTGGCTAGGCAACATAATCATGTTTGCCCCCACACTGTGG
TCTGTGTGAGGTCAGTTAAGGACCGATCCGTTGTATTAGTACAAACGGGGGTGTGACACC

8521 AlaArgMetIleLeuMetThrHisPhePheSerValLeuIleAlaArgAspGlnLeuGlu
GCGAGGATGATACTGATGACCCATTTCTTTAGCGTCCTTATAGCCAGGGACCAGCTTGAA
CGCTCCTACTATGACTACTGGGTAAAGAAATCGCAGGAATATCGGTCCCTGGTCAACTT

8581 GlnAlaLeuAspCysGluIleTyrGlyAlaCysTyrSerIleGluProLeuAspLeuPro
CAGGCCCTCGATTGCGAGATCTACGGGGCCTGCTACTCCATAGAACCACCTTGATCTACCT
GTCCGGGAGCTAACGCTCTAGATGCCCCGGACGATGAGGTATCTTGGTGAACCTAGATGGA

8641 ProIleIleGlnArgLeuHisGlyLeuSerAlaPheSerLeuHisSerTyrSerProGly
CCAATCATTCAAAGACTCCATGGCCTCAGCGCATTTTCACTCCACAGTTACTCTCCAGGT
GGTTAGTAAGTTTCTGAGGTACCGGAGTCGCGTAAAGTGAGGTGTCAATGAGAGGTCCA

8701 GluIleAsnArgValAlaAlaCysLeuArgLysLeuGlyValProProLeuArgAlaTrp
GAAATTAATAGGGTGGCCCGCATGCCTCAGAAAACCTGGGGTACCGCCCTTGCGAGCTTGG
CTTTAATTATCCACCGGCGTACGGAGTCTTTTGAACCCCATGGCGGGAACGCTCGAACC

8761 Gly
ArgHisArgAlaArgSerValArgAlaArgLeuLeuAlaArgGlyGlyArgAlaAlaIle
AGACACCGGGCCCGGAGCGTCCGCGCTAGGCTTCTGGCCAGAGGAGGCAGGGCTGCCATA
TCTGTGGCCCGGGCCTCGCAGGCGCGATCCGAAGACCGGTCTCCTCCGTCCCGACGGTAT

8821 CysGlyLysTyrLeuPheAsnTrpAlaValArgThrLysLeuLys
TGTGGCAAGTACCTCTTCAACTGGGCAGTAAGAACAAGCTCAAAC
ACACCGTTCATGGAGAAGTTGACCCGTCATTCTTGTTCGAGTTTG

FIG. 17-10

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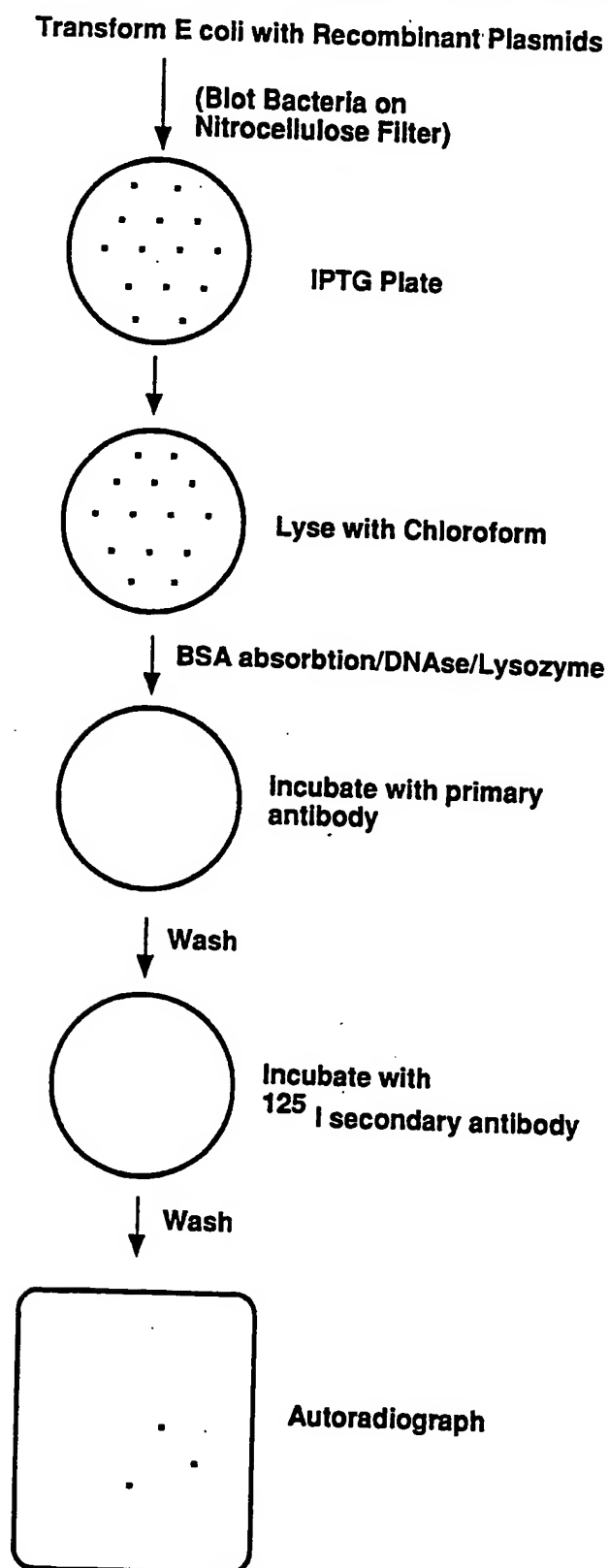
IMMUNOLOGICAL SCREENING IN BACTERIA**FIG. 18****SUBSTITUTE SHEET**

FIG. 19-1

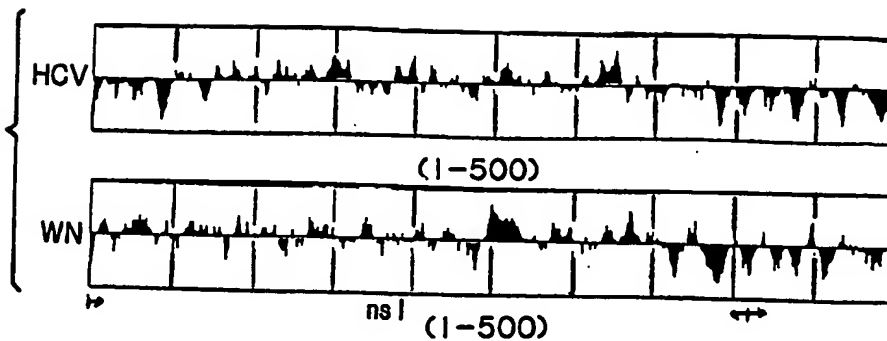


FIG. 19-2

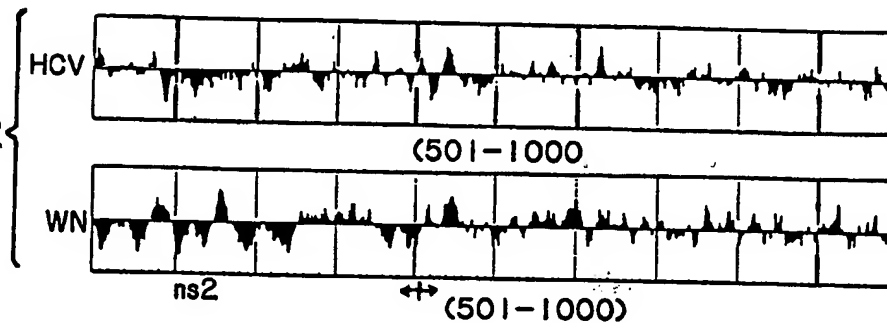


FIG. 19-3

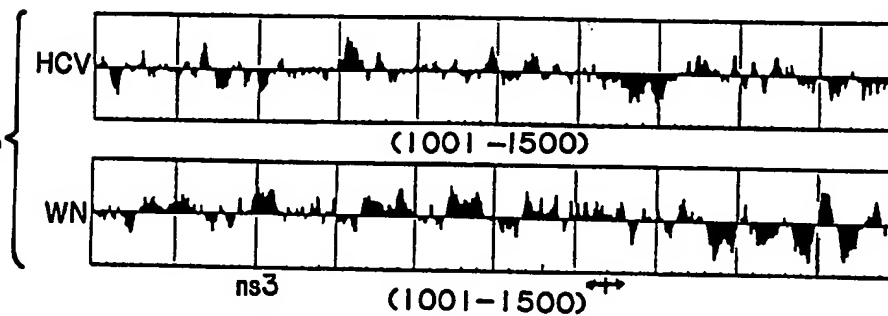


FIG. 19-4

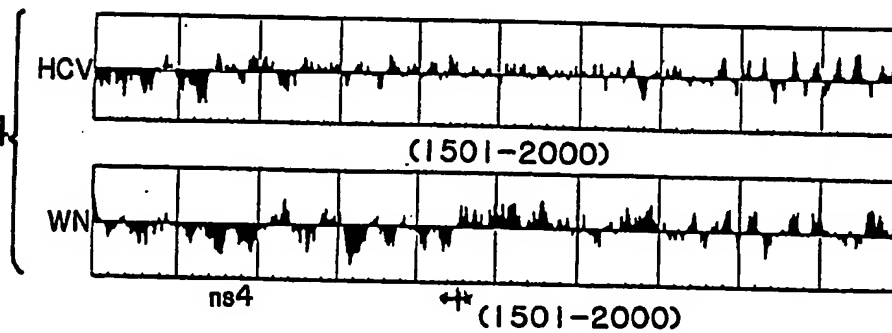
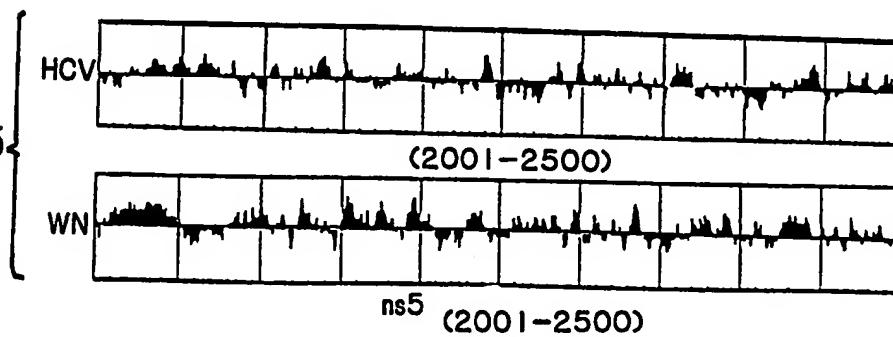
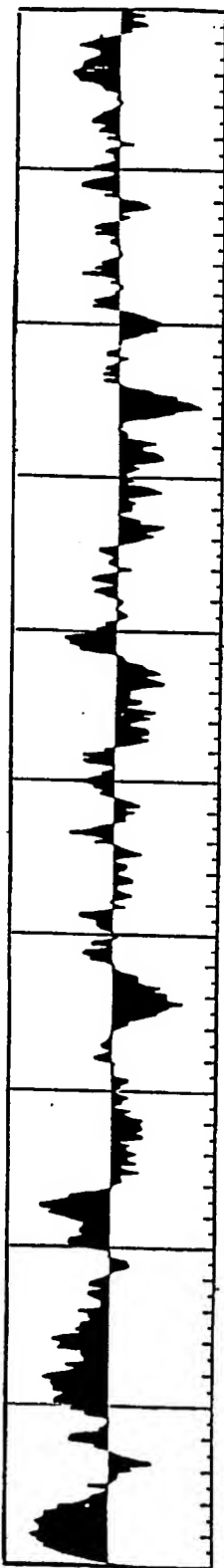


FIG. 19-5



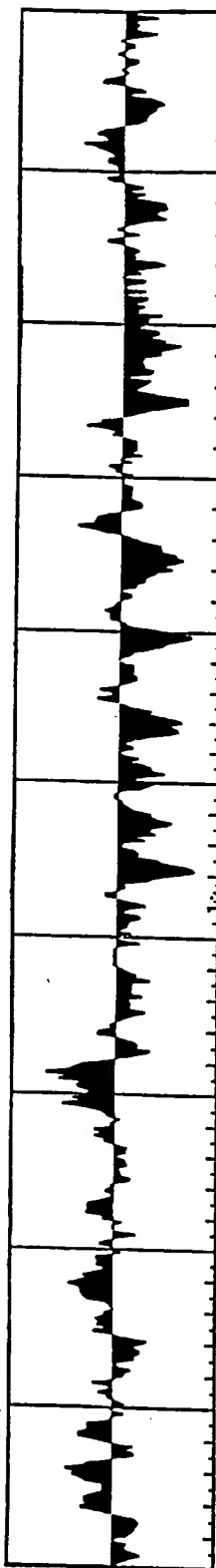
HYDROPHILIC FIG. 20-1



ANTIG INDEX

HCV (1 - 500)

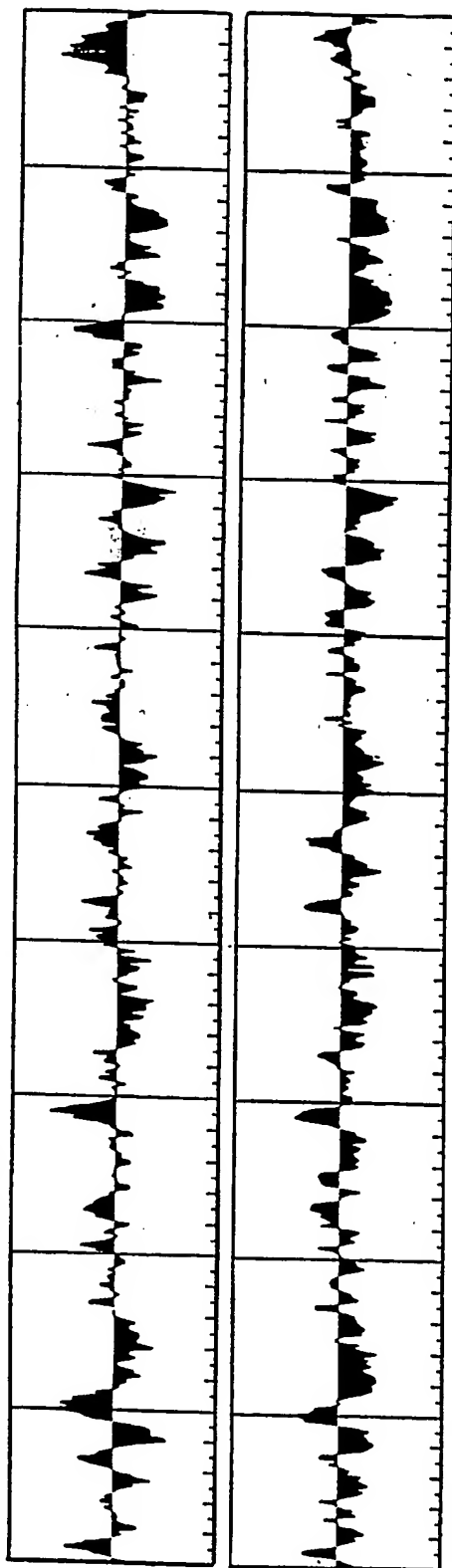
HYDROPHILIC FIG. 20-2



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HCV (501 - 1000)

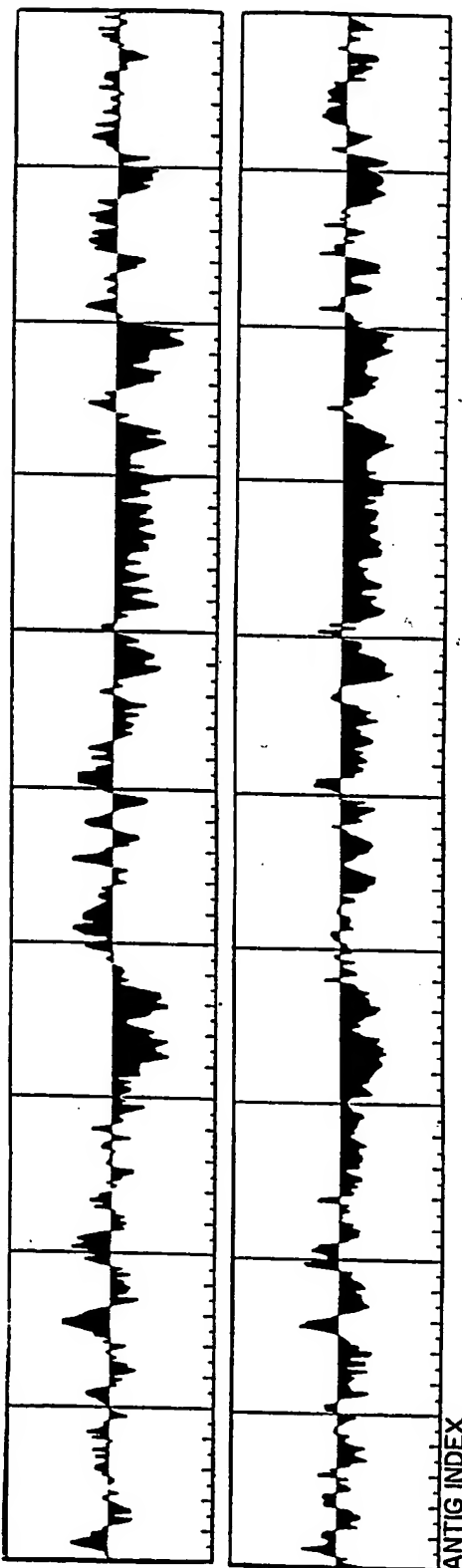
HYDROPHILIC FIG. 20-3



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HCV (1001 - 1500)

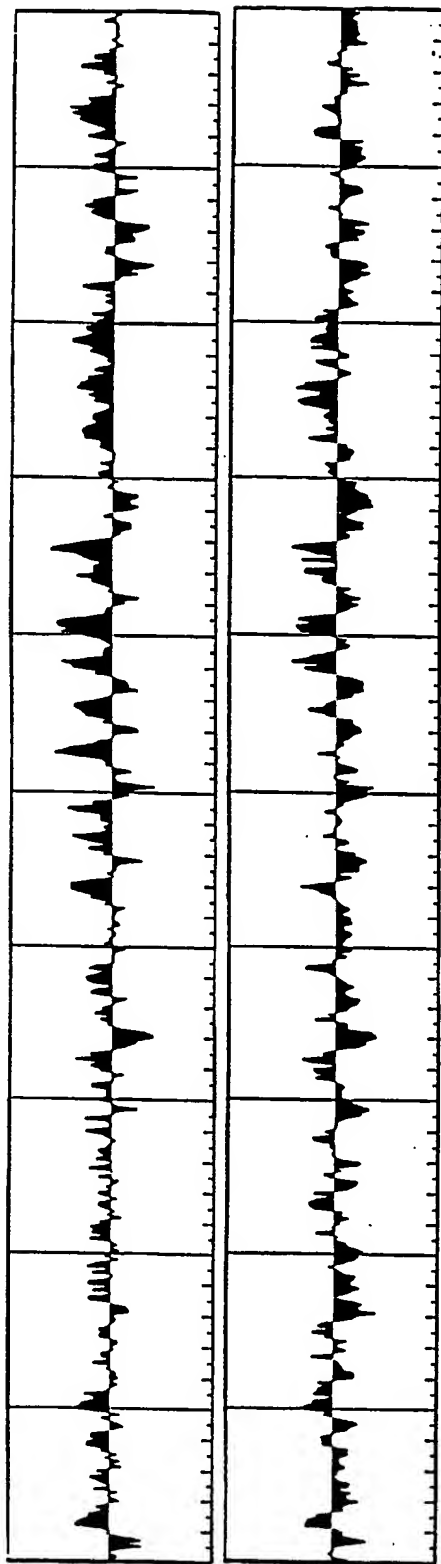
HYDROPHILIC FIG. 20-4



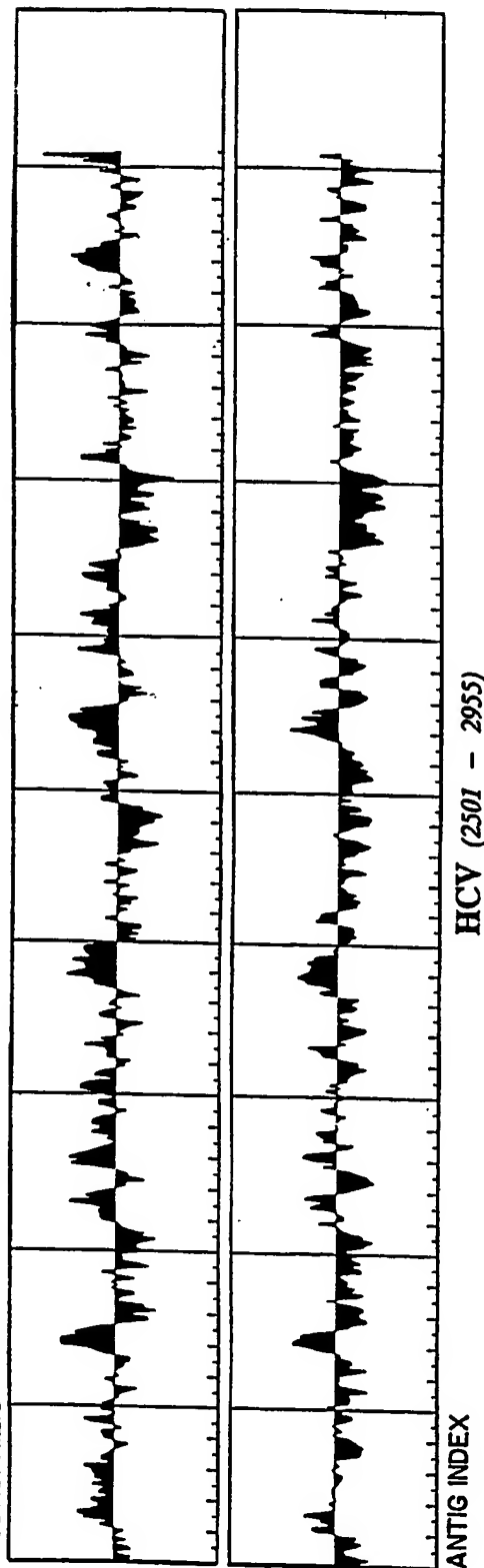
ANTIGEN INDEX

HCV (1501 - 2000)

HYDROPHILIC FIG. 20-5



HYDROPHILIC FIG. 20-6



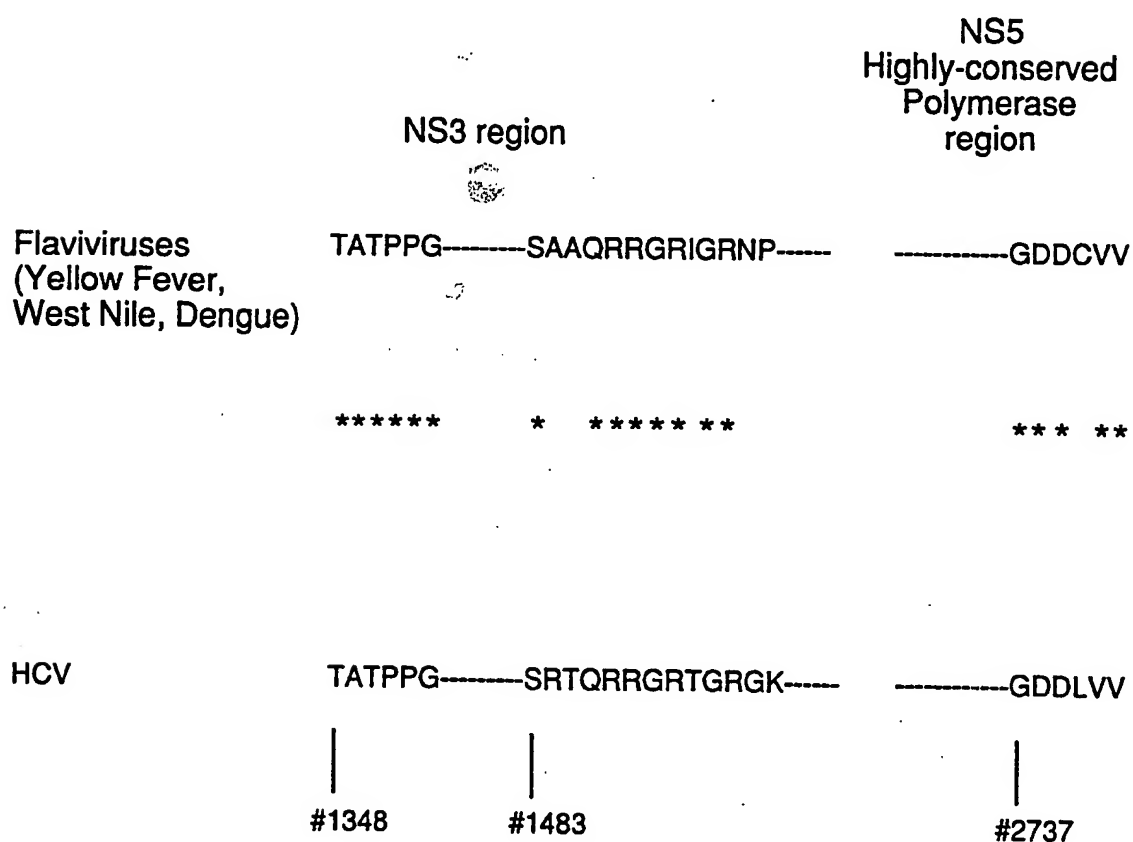
Some conserved co-linear peptides in HCV & Flaviviruses

FIG. 2 I

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US90/01 348

| | | |
|--|---|-------------------------------------|
| I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all) ⁶ | | |
| According to International Patent Classification (IPC) or to both National Classification and IPC | | |
| IPC(5):A61K 39/12;C07H 21/04;C07K 7/06,08,10,13/00,15/28; US: 536/27;435/6,7,69.1,320,240.1;530/324-327,350,387,416;424/89 | | |
| II. FIELDS SEARCHED | | |
| Minimum Documentation Searched ⁷ | | |
| Classification System | Classification Symbols | |
| U.S. | 536/27;435/6,7,69.1,320,240.1 530/324-327,350,387,416; 424/89 | |
| Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched ⁸ | | |
| Automated Patent Search, Chemical Abstract Service | | |
| III. DOCUMENTS CONSIDERED TO BE RELEVANT ⁹ | | |
| Category ¹⁰ | Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹² | Relevant to Claim No. ¹³ |
| A,P | US, A, 4,870,026 WANDS ET AL. 26 September 1989 | 1-34 |
| A | US, A, 4,702,909 VILLAREJOS ET AL. 27 October 1987 | 1-34 |
| A | US, A, 4,542,016 TREPO 17 September 1985 | 1-34 |
| X,P | Science, "Isolation of a c DNA clone derived from a blood-borne non-A, non-B viral hepatitis genome" volume 244, pp 359-362. CHOO ET AL. 21 April 1989 see abstract & figs. | 1-12,15,16,18-20,22,28-31,33 |
| X,P | Science, "An assay for circulating antibodies to a major ehologic virus of human nonA,nonB Hepatitis" volume 244, pp 362-364. KUO ET AL 21 April 1989 see abstract. | 18,19,22 |
| <p>¹⁰ Special categories of cited documents:</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"&" document member of the same patent family</p> | | |
| IV. CERTIFICATION | | |
| Date of the Actual Completion of the International Search | Date of Mailing of this International Search Report | |
| 28 MAY 1990 | 13 JUL 1990 | |
| International Searching Authority | Signature of Authorized Officer | |
| ISA/US | NINA COSSANNA | |

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

Continuation fo Classification:

IPC(5) C12N 1/11,15/02,51 C12P 21/02; C12Q 1/68;
G01N 33/53

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE ¹

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers . . . , because they relate to subject matter ¹² not required to be searched by this Authority, namely:

2. ☐ Claim numbers . . . , because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out ¹², specifically:

3. ☐ Claim numbers . . . , because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING ²

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

- ☐ The additional search fees were accompanied by applicant's protest.
☐ No protest accompanied the payment of additional search fees.